UNIVERSITY OF SOUTHAMPTON

Fetal Programming of the Hypothalamic-Pituitary-Adrenal Axis:
Is cortisol production upregulated centrally in low birthweight individuals?

Alexandra Monica Vivienne Ward
MRCP MBBS

Submitted for the degree of Doctor of Philosophy

MRC Environmental Epidemiology Unit
Fetal Origins of Adult Disease Division
School of Medicine
Faculty of Medicine, Health and Life Sciences
University of Southampton

October 2003

UNIVERSITY OF SOUTHAMPTON
ABSTRACT

FACULTY OF MEDICINE, HEALTH AND LIFE SCIENCES
MRC ENVIRONMENTAL EPIDEMIOLOGY UNIT
Doctor of Philosophy

FETAL PROGRAMMING OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS:
Is cortisol production upregulated centrally in low birthweight individuals?

by Alexandra Monica Vivienne Ward

The 'fetal origins' hypothesis proposes that adverse environmental exposures during critical periods in development result in programmed alterations in physiology and structure that may be disadvantageous to the individual in later life. This idea arose from epidemiological studies linking size at birth with various chronic diseases, particularly cardiovascular disease and its risk factors, and is supported by numerous animal models. In the search for mechanisms to explain the epidemiological observations and given the central role of glucocorticoids in both health and disease, the hypothalamic-pituitary-adrenal axis (HPAA) has been the focus of considerable research interest. Previous studies in humans have shown an inverse relationship between birthweight and both fasting 0900h cortisol concentration and the adrenal response to adrenocorticotrophic hormone (ACTH). The work presented in this thesis was designed to investigate whether these findings are the result of central upregulation of cortisol production in low birthweight individuals.

The first study compared three tests of central HPAA function in a group of low birthweight and high birthweight men aged 60-69 years from Hertfordshire, UK. There were no differences in the salivary cortisol response to awakening or ACTH and cortisol responses during a 100µg corticotrophin-releasing hormone (CRH) test, but low birthweight men had significantly smaller pituitary-adrenal responses during a dexamethasone-suppressed CRH test. While these findings do not explain the HPAA abnormalities associated with low birthweight in previous studies, they provide further evidence of dysregulation of the HPAA in men who were small at birth.

In further analysis of the data, blood pressure, glucose tolerance and plasma lipid concentrations were not related to these measures of central HPAA activity, despite significant positive correlations with morning cortisol concentrations. These data suggest that other mechanisms, for example altered glucocorticoid metabolism, are responsible for elevating circulating cortisol concentrations in men with cardiovascular risk factors.

The second study explored cortisol and blood pressure responses to a series of psychological stress tests in a group of young men and women from Adelaide, Australia. Cortisol responses were not related to size at birth in either sex, but in women there was a significant inverse relationship between birthweight and blood pressure reactivity. This study provides the first human evidence that haemodynamic responses to psychological stress may be programmed antenatally, suggesting a potential mechanism linking reduced fetal growth with raised blood pressure and cardiovascular disease in later life.

In summary, this research does not support the idea that the HPAA is upregulated centrally in low birthweight individuals, but adds to the evidence that the activity of the axis may be influenced by factors affecting fetal growth. Programmed alterations of blood pressure are frequently reported in both human and animal studies and the findings of the stress study should stimulate further research into the mechanisms underlying these observations, particularly alterations in autonomic nervous system function. The work presented in this thesis has added complexity to the role of the HPAA in the fetal origins of adult disease, and confirms that this is likely to remain an exciting area of research in years to come.

Contents   Page

List of Figures

List of Plates

Acknowledgements

Declaration

I declare that this thesis was written by me and that the data presented within it are the result of work carried out in large part by myself.

Specifically, for the Hertfordshire study (Chapter 2) I designed the study, gained ethical approval, recruited the participants, coordinated the study (ordering equipment, booking clinics etc), carried out all the CRH tests and performed the statistical analysis. The new Hertfordshire cohort was assembled by the Hertfordshire Study Group and all the baseline data was collected by them (both questionnaire and lab data). The ACTH and cortisol assays were performed by staff in the Endocrine laboratory at Southampton General Hospital under the supervision of Dr Peter Wood. The dataset was put together by Holly Syddall who has overall responsibility for the Hertfordshire data.

For the Adelaide study (Chapter 3), I designed the study with advice from Professor Andrew Steptoe, put together the questionnaires, set up the study and began recruitment of participants, carried out the stress tests while in Adelaide (35 in total), assembled and cleaned the dataset and performed all the statistical analysis. Ethical approval was gained by Dr Vivienne Moore. While I was away from Adelaide, recruitment and stress testing was continued by Dr Caroline Smith, Meaghan Coyle and Catherine Gibson, though all problems and decisions were referred to me. The salivary cortisol assays were performed by Dr David Kennaway in Adelaide. Data entry was done by Kaye Robinson.

Alexandra MV Ward
2003

Abbreviations

ACTH	Adrenocorticotrophic hormone
AT	Angiotensin
AUC	Area under the curve
AVP	Arginine vasopressin
BMI	Body mass index
CAH	Congenital adrenal hyperplasia
CBG	Corticosteroid-binding globulin
CES-D	Centre for Epidemiologic Studies depression scale
CI	Confidence interval
CRH	Corticotrophin-releasing hormone
CSF	Cerebrospinal fluid
CV	Coefficient of variation
DBP	Diastolic blood pressure
DEX/CRH	Dexamethasone-suppressed CRH test
DST	Dexamethasone suppression test
FPG	Fasting plasma glucose
GR	Glucocorticoid receptor
HAD	Hospital anxiety and depression scale
HBW	High birthweight
HDL	High-density lipoprotein
HOMAβ	Homeostasis model assessment – insulin secretion
HOMAr	Homeostasis model assessment – insulin resistance
HPAA	Hypothalamic-pituitary-adrenal axis
HR	Heart rate

HSD	Hydroxysteroid dehydrogenase
iAUC	Incremental area under the curve
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IQR	Interquartile range
IRSD	Index of relative socio-economic disadvantage
LBW	Low birthweight
LDL	Low-density lipoprotein
MR	Mineralocorticoid receptor
MRC	Medical Research Council
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
OGTT	Oral glucose tolerance test
PEPCK	Phosphoenolpyruvate carboxykinase
PI	Ponderal index
POMC	Pro-opiomelanocortin
PVN	Paraventricular nucleus
SBP	Systolic blood pressure
SD	Standard deviation
SEM	Standard error of the mean
SMR	Standardised mortality ratio
SSTR	Subscapular to triceps ratio
TSST	Trier Social Stress Test
WHO	World Health Organisation
WHR	Waist to hip ratio

CHAPTER 1

Introduction

Cardiovascular diseases are the predominant cause of death in developed countries, accounting for 48% of all deaths in 1990 (1). As the 21st century begins, traditional infectious diseases are declining in the developing world and life expectancy is increasing. These changes in demography are producing dramatic increases in the prevalence of Western diseases, especially when more affluent, sedentary lifestyles are adopted. The Global Burden of Disease Study carried out by the World Health Organisation (WHO) has suggested that by 2020 11.1 million deaths per annum will be attributable to ischaemic heart disease and 7.7 million to stroke (2). In addition, ischaemic heart disease is projected to become the leading cause of disability-adjusted life years; a measure that estimates healthy life years lost due to illness, either through premature death or physical impairment. An ageing global population with its associated increase in chronic disease poses a major challenge to healthcare provision.

Traditionally, it has been thought that cardiovascular diseases arise as a result of an inherited predisposition compounded by a variety of risk factors to which an individual is exposed in adulthood; smoking, elevated lipids and obesity, for example. However, changes in gene frequency cannot explain increased disease incidence over a short time frame and targeting traditional risk factors has proved disappointing in reducing rates of ischaemic heart disease (3). It is of paramount importance that the pathogenesis of these conditions is fully understood in order to target therapies at prevention as, once the disease is established, lasting damage may have taken place.

1.1 FETAL ORIGINS OF ADULT DISEASE

1.1.1 Cardiovascular disease

In the mid 1980s, Barker and colleagues noted that coronary heart disease rates between 1968 and 1978 in England and Wales showed strong correlations with infant mortality rates in the early 20th century (4). As this link could not be explained by traditional risk factors, it was hypothesised that environmental influences during early life, principally fetal undernutrition, might have an impact on health in adulthood.

More detailed epidemiological studies have taken the hypothesis further, using size at birth as an indicator of fetal nutrition. A valuable resource for undertaking such research was discovered in the county of Hertfordshire, UK, where detailed records of all births were kept from 1911 – 1948. From these records, 10,141 men and 5,585 women were traced and deaths between the ages of 20 and 74 were ascertained (5, 6). The standardised mortality ratio (SMR) for coronary heart disease death was inversely related to birthweight in both sexes, as was stroke death in men. Deaths from non-cardiovascular causes were not related to birthweight. The findings of this early study have been replicated in several other populations around the world, from developing as well as developed countries (7-11).

In essence, this work has shown that it is small size at birth, and not simply prematurity, which confers the increased risk of coronary heart disease, that, where other birth measurements are available, it is stunting or thinness at birth that are particularly important and that adult lifestyle factors, such as smoking, alcohol, obesity and social class, do not confound the observations but add to the effects of small size at birth.

1.1.2   Risk factors for cardiovascular disease

The major biological risk factors for cardiovascular disease are hypertension, hyperlipidaemia and impaired glucose tolerance or type 2 diabetes. The relationship between hypertension and size at birth has been examined extensively and the results of eighty studies have been published as a systematic review (12). There is a consistent inverse relationship between birthweight and systolic blood pressure throughout life, although this weakens slightly in adolescence during the growth spurt. Overall, a 1kg increase in birthweight is associated with a 2mmHg fall in systolic blood pressure. As with studies of coronary heart disease, these findings are not confounded by smoking, alcohol intake or obesity and they relate to growth restriction rather than prematurity. Head circumference was the only other birth measure to show a consistent inverse relationship with systolic blood pressure (0.5mmHg/cm). Studies that have looked at diastolic blood pressure have found similar trends although these tend to be weaker.

Recently, doubts about these findings were raised by one of the authors involved in the systematic review. In a reanalysis of the data, it was suggested that publication bias may have contributed to the strength of the combined analysis as associations between birthweight and blood pressure were weaker in larger studies compared with smaller ones (13). However, it is important to note that in the larger studies both birthweight and blood pressure data were often based on self report rather than objective measures and thus some weakening of the association was inevitable due to regression dilution. Another criticism was the exclusion of papers which did not report regression coefficients, though in fact many of these studies did show an inverse association between birthweight and blood pressure.

Relationships between size at birth and lipids have varied in different studies. In 219 men and women in their fifties from Sheffield, UK, there was a strong inverse relationship between abdominal circumference at birth and total cholesterol, LDL-cholesterol and apolipoprotein B (14). Reduced abdominal circumference is thought to reflect fetal undernutrition in late gestation, when the brain is spared at the expense of the liver. The liver plays a central role in lipid metabolism and altered numbers of hepatocytes or receptors may provide the link between size at birth and lipid levels in adulthood. In the Hertfordshire cohort, there were no relationships between total or LDL-cholesterol and birthweight, but in women triglycerides fell with increasing birthweight, while HDL-cholesterol concentrations rose (15).

Associations between size at birth and glucose or insulin metabolism have been reported in over forty studies from around the world. In men from the Hertfordshire cohort, there was a strong inverse relationship between birthweight and 2hr glucose concentrations during an oral glucose tolerance test (OGTT) (16). The odds ratio of having an abnormal glucose tolerance test was over six times higher in those at the bottom of the birthweight range compared with those at the top after correcting for current size. In women, similar patterns were seen for both fasting and 2hr glucose and fasting insulin concentrations (15).

A systematic review of forty-eight studies up to March 2002 detailing the relationship between birthweight and a measure of glucose or insulin metabolism was recently published. Meta-analysis of pooled data was not possible due to significant differences in the way the results were reported in different studies. Thus, the overall direction of the effect of birthweight on various outcomes was presented. Inverse relationships were found in 15 of 25 papers for fasting plasma glucose, 20 of 26 papers for fasting plasma insulin,

4

20 of 25 papers for 2hr glucose (during OGTT), 13 of 16 papers for prevalence of type 2 diabetes, 17 of 22 papers for measures of insulin resistance and 16 of 24 papers for measures of insulin secretion (17). The remainder of the papers showed either no relationship, a positive association or a U-shaped relationship with the various outcomes. The overall weight of evidence suggests that events in utero that result in small size at birth may lead to impaired glucose and insulin homeostasis later in life.

Hypertension, hyperlipidaemia and impaired glucose tolerance tend to associate within individuals, conferring a greatly increased risk of ischaemic heart disease, in what has been termed the metabolic or insulin resistance syndrome (18). In the Hertfordshire cohort, 30% of subjects who weighed 5.5lbs (2.49kg) or less at birth had the metabolic syndrome defined on blood pressure, triglyceride and 2hr glucose concentrations, compared with 6% of those who weighed 9.5lbs (4.31kg) or more (19). These findings have been repeated in Preston, UK, Uppsala, Sweden and San Antonio, US (20, 21). Detailed studies of insulin sensitivity support these observations. Using the euglycaemic hyperinsulinaemic clamp technique, McKeigue and colleagues showed that insulin sensitivity improved with increasing birthweight in a large cohort of Swedish men (22). Other groups have used alternative methodology (short insulin tolerance test or intravenous glucose tolerance test with minimal model analysis) and also found that low birthweight is associated with insulin resistance (23, 24).

In summary, there is now considerable epidemiological evidence to support the 'fetal origins' hypothesis. Principally, events in early life that result in reduced fetal growth appear to have lasting health implications for the individual. These observations have opened up a new avenue of research into the pathogenesis of cardiovascular and metabolic

diseases. While the majority of interest has centred on these conditions, there is also evidence that similar factors may have a role in the aetiology of osteoporosis, depression and possibly some malignancies (25-27). Detailed discussion of these topics is beyond the scope of this thesis.

One possible explanation of the associations described above is that genes which restrict fetal growth may also result in adult disease. For example, insulin is a major growth factor in utero and it has been suggested that genes which confer insulin resistance, and thus promote the development of type 2 diabetes, could also result in fetal growth retardation leading to a spurious association between birthsize and later diabetes (28). However, in contradiction of this hypothesis, a study in monozygotic twins discordant for type 2 diabetes found that the affected sibling had a significantly lower birthweight (2.63 vs 2.83 kg, p<0.02) (29). The following section expands the evidence that environmental exposures may be important in the fetal origins of adult disease.

1.2 PROGRAMMING

Many organisms show plasticity during critical periods in their development rendering them sensitive to external stimuli. During these windows, the organism may be 'programmed' by its environment, resulting in permanent alteration of structure or resetting of physiological systems. Such adaptations may be disadvantageous to the organism if it subsequently encounters a different environment. Thermoregulation provides an example of this phenomenon in humans. Babies are unable to sweat immediately after birth, but glands become active in infancy. In the first half of the 20th Century, Japanese physiologists showed that while all individuals possess a similar number of sweat glands in a given area of skin, the number of functioning glands varies. They found that final number of functional glands is dependent on the prevailing temperature during the first three years of life and is thereafter fixed, explaining why some individuals find it difficult to tolerate moving to a hotter climate later in life (30).

The idea that programming might underlie the epidemiological observations described in section 1.1 has led to the development of animal models in which specific insults and windows of vulnerability can be examined in greater detail.

1.2.1 Fetal programming of hypertension

Models have been developed in a number of species to explore the mechanisms underlying the inverse association between fetal growth and blood pressure. Many of these focus on nutrient restriction or imbalance, as proposed by the 'fetal origins' hypothesis. In rats, global nutrient restriction, protein restriction, iron deficiency and excess fat during pregnancy have all been shown to increase blood pressure in exposed offspring compared with controls (31-36). In most of these studies, the dietary manipulation resulted in

reduced birthweight. Blood pressure differences persisted throughout life and, where examined, appeared to amplify with age (33). Gender differences have been identified in some studies; in rats exposed to a 30% reduction of maternal intake during pregnancy, male offspring were more affected than females (33), while exposure to a high fat diet caused hypertension in the female offspring alone (36). The mechanisms underlying these gender differences are not yet understood.

Maternal undernutrition has also been examined in guinea pig and ovine models. A 15% reduction of food intake throughout pregnancy elevated blood pressure in three month old male guinea pig offspring. In addition, an inverse association between birthweight and blood pressure was seen in the control animals in this study (37). Young lambs exposed to 15% feed restriction during the first 70 days of gestation had increased basal mean arterial pressure compared with controls (38). Another group have shown that a 50% reduction in food intake in sheep for the last 30 days of pregnancy increased blood pressure in the fetuses during the intervention, but the postnatal outcome of these offspring has not been published (39).

Nutrient restriction can also be achieved by limiting placental supply to the developing fetus. In a rat model using uterine artery ligation, there were no relationships between birthweight and either resting or stressed blood pressures (40), but a similar study in guinea pigs did elevate of blood pressure in severely growth-retarded offspring (41). In sheep, placental supply can be restricted by reducing the number of uterine caruncles prior to pregnancy or by embolizing part of the placenta in late gestation. Both of these models have been shown to alter cardiovascular regulation in late fetal life (39).

Antenatal administration of glucocorticoid hormones, which aims investigate the effect of therapy for preterm labour and to mimic the stress response induced by maternal undernutrition, is another frequently used model. These studies are covered in detail in section 1.4.1.3. Briefly, fetal exposure to excess glucocorticoid has been shown to increase blood pressure in both rats and sheep, though the same effect is not seen in guinea pig models (42-44).

1.2.2   Fetal programming of glucose tolerance

Fewer studies have focussed on programmed alterations in glucose/insulin homeostasis. A large body of work has been published by Hales and colleagues. Their rat model involves 50% protein restriction during pregnancy and lactation. Offspring have better glucose tolerance and insulin sensitivity than controls as young adults, but then experience an accelerated decline in insulin sensitivity with age, ultimately rendering them less glucose tolerant. These researchers have also demonstrated an interaction between prenatal insults and post natal environment. If the protein-restricted offspring become obese as adults, as a result of increased food intake, they develop many features of the metabolic syndrome (45). It is noteworthy that blood pressure is not altered in this model, despite a similar degree of protein restriction to that used in the hypertension model extensively researched by Langley-Evans and colleagues (46). It is likely that the altered balance of nutrients in these two diets, resulting from different carbohydrate and fat sources, is responsible for the distinct programming phenomena observed, highlighting the specificity of different antenatal insults.

In other work, a recent study showed that moderate (30%) feed restriction in utero rendered young male guinea pigs hyperinsulinaemic, though glucose tolerance was not

altered. Similar abnormalities were not found in the female offspring in this model (47).

Glucose tolerance has also been found to be abnormal in rats exposed to glucocorticoids

antenatally (see section 1.4.1.3) (48, 49).

1.2.3 Mechanisms underlying fetal programming of adult disease

The animal models described above support the observations in human epidemiological

studies linking antenatal events with disease in later life. They also allow detailed

investigation of the possible underlying mechanisms. Many factors involved in the control

of blood pressure and glucose tolerance have been shown to be altered in offspring

exposed to these prenatal insults. Examples include structural changes (reduced nephron

and β cell number), altered baroreflex settings, vascular dysfunction (impaired

vasodilatation) and resetting of enzyme systems (enhanced gluconeogenesis) (45, 50).

A number of hormonal systems (hypothalamic-pituitary-adrenal, renin-angiotensin,

sympatho-adrenal, growth hormone/insulin-like growth factor-1) are directly involved in

blood pressure regulation and metabolism, making them important targets for research in

this field. As a result, evidence is accumulating that the activity of these systems may

programmed by antenatal insults (51).

The remainder of this introduction considers the hypothalamic-pituitary-adrenal axis and

the sympathoadrenal system and the role they may play in the fetal origins of adult

disease.

1.3 OVERVIEW OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

The hypothalamic-pituitary-adrenal axis (HPAA) controls the release of glucocorticoid hormones; principally, cortisol in humans, guinea pigs and sheep and corticosterone in rodents. Glucocorticoids are catabolic hormones which act to mobilise fuel in times of stress, antagonising the principal actions of insulin. In the liver, glucocorticoids enhance gluconeogenesis via upregulation of the activity of the rate-limiting enzyme phosphoenolpyruvate carboxykinase (PEPCK) while, in skeletal muscle, they reduce translocation of GLUT4 transporters to the cell surface. In adipose tissue, glucocorticoids stimulate lipolysis and reduce the activity of lipoprotein lipase (52). They are also important regulators of salt and water homeostasis and immune function and they influence behaviour, cognition and memory formation.

1.3.1 Organisation of the HPAA

The basic structure of the HPAA is outlined in Figure 1.1. The principal stimulus to glucocorticoid release from the adrenal cortex is adrenocorticotrophic hormone (ACTH). ACTH is cleaved from pro-opiomelanocortin (POMC) and released from corticotrophs in the anterior pituitary in response to corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP). These hormones are secreted into the hypophysial portal circulation by cells in the parvocellular region of the paraventricular nucleus (PVN) of the hypothalamus. There are numerous inputs to the hypothalamus from different brain regions, acting via a myriad of neurotransmitters; particularly important are those from the hippocampus which are predominantly inhibitory and those from the amygdala and brainstem which are excitatory (53).

Figure 1.1 The hypothalamic-pituitary-adrenal axis.

Stress

Limbic system

Neurotransmitters

**Hypothalamic
PVN**

**CRH
(+AVP)**

**Anterior
pituitary**

ACTH

**Adrenal
cortex**

Cortisol

Stimulatory

Inhibitory

$\triangle$ GR

$\blacktriangle$ MR
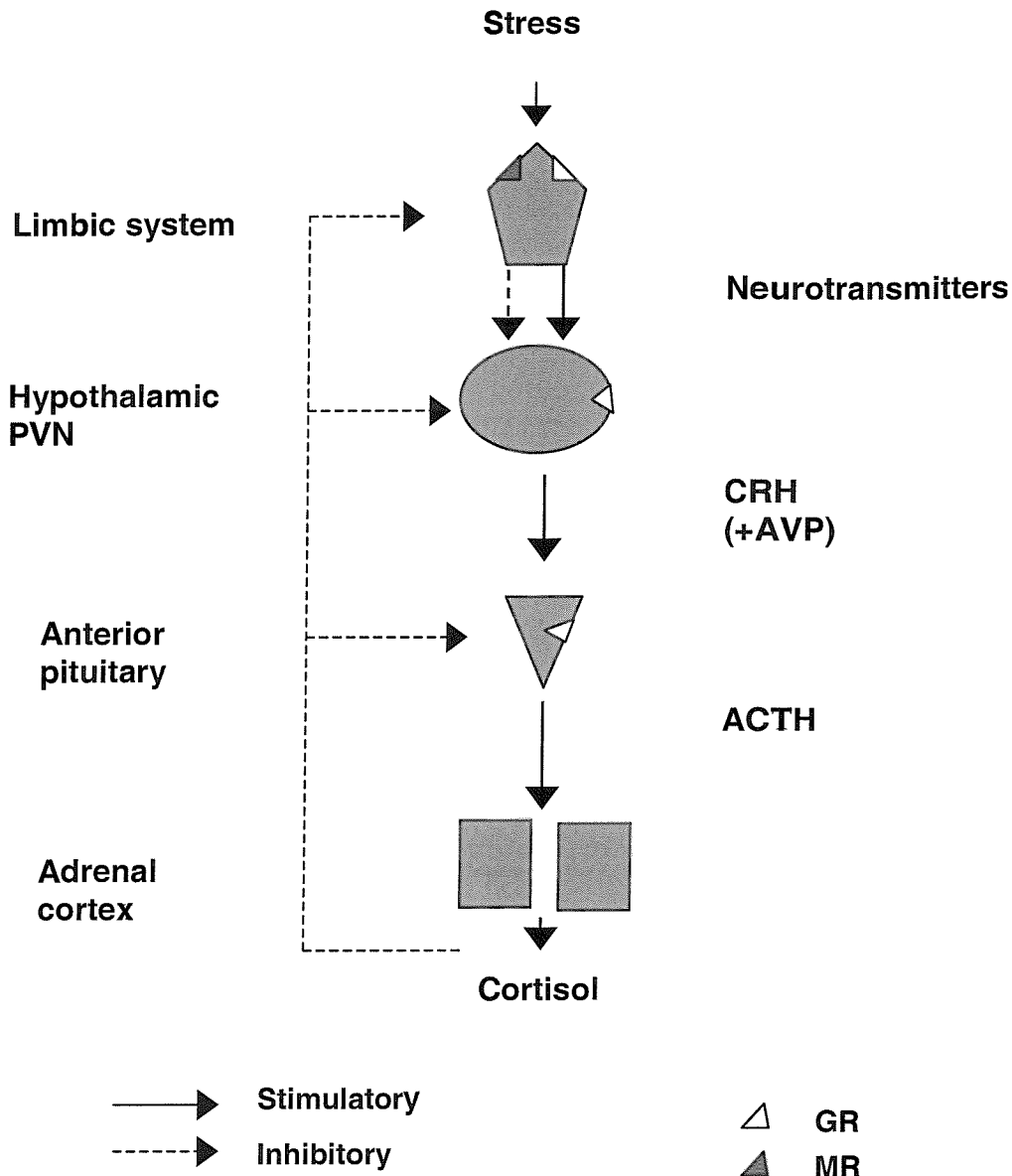
Afferent fibres from a number of different brain regions feed into the hypothalamus, resulting in either stimulation or inhibition. Noradrenaline, acetylcholine and 5-HT are the principal stimulatory neurotransmitters, while GABA is inhibitory. Following stimulation, CRH and to a lesser extent AVP are synthesised in the parvocellular region of the hypothalamic paraventricular nucleus (PVN). These hormones act on corticotrophs in the anterior pituitary to cleave pro-opiomelanocortin (POMC) releasing ACTH which then circulates to the adrenal cortex to stimulate the production of cortisol. The activity of the HPAA is controlled by negative feedback; cortisol acts at glucocorticoid receptors (GR) throughout the axis and mineralocorticoid receptors (MR) in the hippocampus.

1.3.2 Tissue availability and activity of glucocorticoids

Two mechanisms control access of glucocorticoid hormones to their receptors. In the circulation, glucocorticoids are largely bound to corticosteroid-binding globulin (CBG) and only the free hormone (10%) is able to pass into cells. CBG is part of the serine protease inhibitor family of proteins and is cleaved by neutrophil elastase, reducing its binding affinity and thus possibly targeting delivery of glucocorticoid to sites of inflammation (54). Within cells, the amount of active glucocorticoid is controlled by the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD). This enzyme interconverts cortisol and inactive cortisone (corticosterone and 11-dehyrocorticosterone in rodents) and exists in two isoforms; 11β-HSD1 is widely expressed and bidirectional, but acts principally as a reductase (reactivating cortisol), and 11β-HSD2 is unidirectional (cortisol to cortisone) and is only found in the kidney, colon, pancreas, placenta and gonads (55, 56). Glucocorticoids act via two intracellular receptors; high affinity mineralocorticoid receptors (MR) have a limited tissue distribution (kidney, colon, hippocampus) and lower affinity glucocorticoid receptors (GR) are widespread throughout the body. After dimerization within the cytoplasm, the hormone-receptor complex is translocated to the nucleus where it influences the transcription of glucocorticoid-sensitive genes.

1.3.3 Regulation of the HPAA

The activity of the HPAA follows a tight circadian rhythm under the control of the suprachiasmatic nucleus which has direct input into the PVN. In humans, HPAA activity peaks in the morning and falls to a nadir around midnight, while in rodents the pattern is reversed. Stress-induced surges are superimposed on this rhythm. Direct stimulation of the PVN by ascending noradrenergic neurones from the brainstem and locus coeruleus links activation of the HPAA closely to that of the sympathoadrenal system and occurs

predominantly in response to somatic stimuli, such as haemorrhage. Psychological stimuli are processed in the limbic system and activate the HPAA principally via the amygdala, which is responsible for coordinating the behavioural responses to fear and anxiety. Situations which result in feelings of helplessness, unpredictability or lack of control are strong stimuli to the HPAA (57). Other stimulatory inputs to the PVN may include serotinergic neurones from the raphe nucleus and cholinergic afferents, though the specific roles of these factors are less clear (53).

Inhibition of stress-induced HPAA activity is essential to prevent overexposure to glucocorticoids and is achieved by both hormonal and neuronal mechanisms. Glucocorticoids feed back directly onto GRs in the pituitary and hypothalamus to reduce the release of ACTH and its secretagogues. The most important central inhibitory input into the PVN, acting via GABA-ergic neurones, comes from the hippocampus which expresses high levels of both GR and MR (58). Under basal conditions, with low circulating levels of glucocorticoid, MRs regulate the tone of the axis. At the diurnal peak and during stress, GR occupancy rises rapidly thereby halting CRH synthesis and release, which leads to a reduction in ACTH and cortisol concentrations.

1.3.4   Ontogeny of the HPAA

Development of the HPAA differs between species. In precocial species such as humans, sheep and guinea pigs, the axis is fully developed and largely mature at birth, whereas in rats significant maturation occurs after birth. In humans, the hypothalamus and pituitary are formed by the end of the first trimester and both CRH and POMC mRNA can be identified during the first half of the second trimester (59). Glucocorticoid receptors develop at the same time. In fetal life, glucocorticoid levels are low but in many species

rise towards term when they initiate tissue maturation in preparation for extrauterine existence. It is well established that the fetal HPAA responds to stressful stimuli. In late ovine gestation (day 135), experimental fetal hypoxia inducing a 40% reduction in fetal arterial PO_2 produced robust increases in fetal plasma ACTH and cortisol, which appeared to be mediated by release of CRH and AVP into the hypophysial portal system (60).

1.3.5 The HPAA and the metabolic syndrome

Cortisol excess, as seen in patients with Cushing's syndrome, is characterised by central obesity, hypertension and glucose intolerance among other features. The similarity between this condition and the metabolic syndrome has led to the suggestion that milder dysregulation of HPAA activity may underlie the metabolic syndrome or its components (61). Various mechanisms have been proposed, including increased cortisol secretion, impaired peripheral metabolism of cortisol and enhanced tissue sensitivity to glucocorticoids.

Several large cross sectional studies have examined the relationship between circulating cortisol concentrations and cardiovascular risk factors. In 1528 men from the Paris Prospective Study cohort, there was a positive association between systolic blood pressure and fasting cortisol (62). Walker and colleagues showed that cortisol and obesity were independent predictors of blood pressure in men and of serum triglyceride and fasting plasma insulin in women in the MONICA cohort (63). A significant association between cortisol and fasting insulin was also found in elderly women from the Rotterdam study (64). Bjorntorp and colleagues measured salivary cortisol over the course of a working day in a large group of middle-aged men. In a series of complicated analyses, they showed that

perceived stress-weighted salivary cortisol concentrations were increased in some obese men with cardiovascular risk factors (65).

Similar relationships exist in other ethnic groups; strong positive correlations between blood pressure and glucose tolerance and fasting cortisol were found in a group of middle aged men and women from Mysore, South India (66) and between blood pressure and cortisol in Jamaican children aged eight (67).

Longitudinal studies are needed to fully implicate the HPAA in the pathogenesis of the metabolic syndrome, but these observations have led many researchers to investigate the possibility that programming of HPAA activity might have a role in the link between fetal growth and adult disease, particularly the metabolic syndrome. This hypothesis is supported by data from animal models and recent epidemiological studies in humans. The results of these investigations are detailed in the following section.

1.4 PROGRAMMING OF THE HPAA

1.4.1 Evidence from animal experiments

It has been recognised for several decades that manipulations in early life can have long lasting effects on the HPAA (68). Early experiments into the mechanisms underlying these observations were carried out in neonatal rats, utilising the relative immaturity of the axis after birth in this species. Pups separated from their mothers for fifteen minutes per day in the first three weeks of life, so called neonatal handling, were found to have reduced stress responsiveness throughout life (69) and this was associated with enhanced expression of GR in the hippocampus (70). Longer periods of separation (180 min/day) had the opposite results; long term hyperactivity of the HPAA associated with increased hypothalamic CRH (71). The remainder of this section focuses on antenatal programming of the HPAA.

1.4.1.1 Prenatal stress

The effects of prenatal stress, both psychological (restraint, unpredictable noise and light) and physical (alcohol, immune challenge), have been widely studied in rat models. In general, offspring show enhanced HPAA responses to stress, with or without changes in basal glucocorticoid secretion (72-75). Alongside these changes in HPAA function, alterations in behaviour are often seen; prenatally stressed offspring demonstrate increased anxiety or emotionality in aversive situations. Some results have been contradictory, but the wide range of prenatal stimuli used and differences in the timing of the insult make direct comparison of studies difficult. Indeed, timing is critical; an insult at one stage of gestation that induces a marked change in HPAA function may have no effect at a different time (76). This is likely to relate to the stage of development of the axis at the point of intervention. The age at which offspring are tested also influences the HPAA response. Immature rats are normally hyporesponsive to stress, but this is consistently

abolished by prenatal stress. Gender differences in the effect of prenatal stress have been noted in several studies, with female offspring tending to show more pronounced alterations of HPAA function than males. In one study, prenatally-stressed females showed exaggerated ACTH and corticosterone responses to stress, whereas males showed reduced responses (77).

A guinea pig model has been developed to investigate the effects of an unstable social environment during development. Pregnant dams were housed in groups of five animals and the composition of the group was changed every three days during pregnancy and lactation by moving two dams. Control animals were left undisturbed during this time. This intervention had striking behavioural and endocrine effects, which were clearly sex specific. Female offspring showed masculinisation of certain behaviour patterns, associated with elevated circulating testosterone concentrations. Cortisol concentrations did not differ from controls, but adrenal weight and tyrosine hydroxylase activity were elevated, suggesting enhanced stress responsiveness (78). Conversely, male offspring showed behavioural infantilisation, delayed maturation of the HPAA and reduced sympathoadrenal activity (79).

Schneider and colleagues have performed similar studies in rhesus monkeys. Offspring born to mothers exposed to repeated mild stress (unpredictable noise) in mid to late gestation have reduced birthweight, impaired neuromotor development, attention deficits, increased disturbance behaviour in stressful conditions and altered basal and stress-induced HPAA activity (80). In a follow up study, they have shown that the effects of the same stress in early gestation produced even greater differences in birthweight and motor

18

function, suggesting that sensitivity to stress in this species is greater in early gestation (81).

Several groups have looked at the expression of glucocorticoid receptors in relation to prenatal stress exposure. In adult rats, reduced MR and GR densities have been found in the hippocampus, which may result in impaired feedback control and enhanced HPAA reactivity (72). Again, these findings vary between studies and gender differences have been demonstrated (73, 77).

1.4.1.2 Maternal undernutrition

Maternal undernutrition is a specific prenatal stressor that has been shown to program hypertension and glucose intolerance in adult rat offspring (see section 1.2). In their low-protein diet model, Langley-Evans and colleagues have shown that development of hypertension in the offspring can be blocked by treatment of the mothers with metyrapone, to inhibit corticosterone synthesis, and is dependent on an intact HPAA in the offspring (82, 83). This suggests that glucocorticoids may be responsible for both programming and maintaining hypertension in this model. Examination of the HPAA for evidence of programmed alterations in activity revealed a blunted ACTH diurnal rhythm in weanling rats, but no difference in corticosterone secretion between the prenatally undernourished and control groups. However, two studies have suggested that glucocorticoid action is increased in this model. Glucocorticoid-inducible enzyme activity was elevated in brain (83) and Bertram and colleagues found increased GR mRNA and protein in a number of classical glucocorticoid target tissues (kidney, liver, lung), along with reduced 11β-HSD 2 expression in the kidney and adrenal (84). These abnormalities persisted throughout the

life course of the protein-restricted offspring. GR expression in the hypothalamus was reduced and there were no differences in 11β-HSD 1 or MR expression in this study.

A further mechanism by which glucocorticoid exposure may be programmed has recently been demonstrated. 50% restriction of total food intake in the third week of gestation and throughout lactation produced a significant reduction in CBG binding capacity in adult male rats compared with controls. Whilst there were no differences in basal levels of CRH, ACTH or corticosterone, free corticosterone concentrations were higher in the nutrient-deprived group after restraint stress (85). No assessment of cardiovascular or metabolic status has been made in these offspring.

In guinea pigs, maximal brain growth occurs in the latter stages of gestation and is associated with rapid development of neuroendocrine systems. This equates with human development, whereas in rats maximal brain growth occurs after birth (86). Complete food deprivation for 48hrs on days 50 and 51 of gestation (term = 70 days) activated the maternal HPAA and fetal cortisol levels rose as a consequence (87). Fetuses sacrificed at this point had reduced GR mRNA in the hypothalamus and hippocampus compared with controls, but there were no differences in MR mRNA content. In adult male offspring, maternal nutrient restriction resulted in reduced basal and stimulated HPAA activity. The opposite effect was found in females, in whom a reduction in GR mRNA levels in the pituitary was also seen (88). Interestingly, there was no effect on blood pressure in either group.

In chronically catheterised sheep fetuses, 15% nutrient restriction for the first 70 days of gestation (term = 147 days) did not alter basal ACTH and cortisol concentrations in late

fetal life, but pituitary and adrenal responses following endogenous (hypoxia) and exogenous (CRH+AVP or ACTH) stimulation were reduced (89, 90). These fetuses also had lower blood pressure than those in the control group, with resetting of the baroreflex. However, when the offspring were studied as young lambs (Day 85) the findings were reversed; baseline mean arterial blood pressure was raised and blood pressure, ACTH and cortisol responses to CRH+AVP were greater than controls (38).

A recent study has shown alterations in the expression of GR and 11β-HSD2 mRNA, similar to those found in the low-protein rat model, in neonatal sheep whose mothers were nutrient restricted during the period of maximal placental growth (Days 28-77), suggesting that tissue exposure to glucocorticoid may also be programmed by maternal undernutrition in sheep (91). Interestingly, these findings were associated with an increase in angiotensin 1 (AT1) receptor expression in the same tissues. This receptor has a glucocorticoid-sensitive gene, highlighting a potential mechanism linking fetal undernutrition to later hypertension via the HPAA.

1.4.1.3   Glucocorticoid exposure

Maternal glucocorticoid is secreted in higher concentrations during stress or undernutrition and may be responsible for programming the developing fetal HPAA (39, 92). The exaggerated stress-induced rise in corticosterone in rats whose mothers had experienced restraint stress in the final week of pregnancy was not seen when the mother had previously undergone adrenalectomy to remove endogenous corticosterone secretion, but was mimicked when an excessive replacement dose of corticosterone was used post adrenalectomy (93). MR expression in the hippocampus followed this pattern reciprocally;

reduced levels were found in the offspring with exaggerated stress responsiveness while no change was found in those of mothers who had undergone adrenalectomy.

Most studies examining the programming effects of antenatal glucocorticoid exposure have utilised the synthetic steroid dexamethasone which is not inactivated by placental 11β-HSD2 and so passes freely to the fetus. It is important to recognise that in this model circulating endogenous glucocorticoid concentrations in the fetus will be reduced by the powerful inhibitory effects of dexamethasone on the HPAA. Thus, the mechanisms underlying the programmed alterations in HPAA activity may differ from those seen following prenatal stress.

Administration of dexamethasone (100μg/kg) to pregnant rats during the final week of gestation reduced birthweight, increased basal but not stress-induced corticosterone levels and reduced hippocampal MR and GR expression (94). These offspring were also hypertensive, but did not have altered GR or MR expression in areas of the brain thought to be directly involved in blood pressure control. Similar experiments have shown that antenatal dexamethasone also programs glucose intolerance, associated with increased GR and PEPCK mRNA levels in the liver (49). Abnormal behaviour, like that seen following prenatal stress, has also been reported in this model and is possibly related to increased CRH mRNA expression in the amygdala (95). Other groups have studied the effect of antenatal glucocorticoid in rat models, but as with prenatal stress the timing and dose of dexamethasone used influences both the qualitative and quantitative effect on the offspring (96).

Guinea pigs show a tenfold reduction in sensitivity to the effects of dexamethasone compared with rodents, reflecting differences in GR affinity (97). Dexamethasone (1mg/kg), given during the period of maximal brain growth in a similar protocol to the maternal nutrient restriction studies described above, has sex-specific effects on the HPAA. In fetal guinea pigs sacrificed the day after dexamethasone exposure, plasma cortisol concentrations were suppressed in males and elevated in females (98). The female fetuses also had alterations in GR and MR mRNA expression in specific regions of the hippocampus. These differences persisted into adulthood. Males had impaired basal and stimulated HPAA activity in association with increased hippocampal MR mRNA. The picture was more complicated in female offspring who showed varying results depending on the phase of the reproductive cycle, suggesting an interaction with sex steroids (99).

The effects of antenatal glucocorticoid exposure have also been investigated in sheep. Sloboda and colleagues examined basal and stimulated (CRH/AVP) HPAA activity in sheep at six and twelve months of age, following a variety of prenatal treatments. None of the exposures affected HPAA function at six months. A single maternal injection of betamethasone (which also crosses the placenta freely) increased basal and stimulated cortisol concentrations without an accompanying difference in ACTH, suggesting that adrenal responsiveness at one year of age is enhanced by this treatment. However, direct administration of betamethasone into the fetal compartment attenuated ACTH responses to CRH/AVP, while cortisol responses were unchanged. Repeated administration of glucocorticoid, either to mother or fetus did not alter HPAA activity compared with controls, but did appear to delay the maturation of the axis between the two ages studied (100). This complicated study serves to highlight the importance of the timing and route of

administration of glucocorticoid and the age at which the offspring are studied in the effects on the HPAA.

Brief dexamethasone administration to pregnant ewes in early gestation (48h, D27) has been shown to reliably induce hypertension in the offspring from four months to seven years of age. The role of the HPAA in this model was investigated in female offspring by exposing them to haemorrhage stress. There were no significant differences in ACTH, AVP or cortisol responses to haemorrhage compared with controls and there were also no differences in corticosteroid receptor expression in the hippocampus or hypothalamus (101).

Dose-dependent abnormalities of hippocampal neurone development have been shown to result from dexamethasone administration to rhesus monkeys in the early third trimester (102). At term, the hippocampal formation was reduced in size, containing 30% fewer neurones, and a similar reduction in volume was found on magnetic resonance imaging (MRI) at twenty months. In addition, animals examined at nine months of age had elevated basal and stress-induced cortisol levels, suggesting that glucocorticoid feedback was impaired in association with the changes seen both macroscopically and microscopically in the hippocampus.

Placental 11β-HSD2 acts as a barrier, limiting the access of maternal glucocorticoid to the fetal circulation. Carbenoxolone, a drug which inhibits this enzyme, has been shown to mirror the effects of prenatal dexamethasone administration in rats. Offspring of mothers treated throughout pregnancy with carbenoxolone were 20% lighter at birth and had high blood pressure and impaired glucose tolerance as adults, findings that were abolished by

maternal adrenalectomy (48, 103). Studies of the HPAA in these offspring have revealed higher basal corticosterone concentrations, reduced GR mRNA in the PVN and increased GR mRNA in the amygdala, associated with enhanced anxiety-like behaviour (104). There were no changes in corticosteroid receptor expression in the hippocampus.

Several groups have investigated the possibility that variations in the activity of 11β-HSD2, with consequent differences in fetal exposure to maternal glucocorticoid, could link reduced fetal growth and later disease. The results of these studies have not been entirely reproducible, but a positive correlation between birthweight and placental 11β-HSD2 activity has been found in both rats (42) and humans (105). In addition, maternal nutrient restriction in rats and sheep has been shown to downregulate placental expression of the enzyme, again implicating glucocorticoids in the long term consequences prenatal undernutrition (84, 91, 106).

1.4.2 Evidence from human studies

1.4.2.1 Antenatal insults

The effects of prenatal stress in humans have been studied retrospectively in groups who have suffered adverse events in pregnancy. There is some evidence for behavioural deficits in childhood, but no detailed studies have been performed to examine stress-responsiveness or HPAA activity in these children and there are no similar studies in adults (76).

Similarly, there is little follow up information on children whose mothers were treated with glucocorticoids during pregnancy. There are two main indications for such treatment. Glucocorticoids are administered in preterm labour to speed fetal organ maturation. In

these children, it is difficult to dissect behavioural disturbance due to fetal glucocorticoid exposure from that due to prematurity. One follow up study of preterm deliveries found that children whose mothers had received a single dose of betamethasone antenatally had higher systolic and diastolic blood pressures at age fourteen than those who had not (107). In congenital adrenal hyperplasia (CAH), dexamethasone is used to protect female fetuses from masculinisation. A pilot study looking at the effects of dexamethasone given in early pregnancy because of an increased risk of CAH showed higher emotionality, unsociability, avoidance and behavioural problems in the exposed children (108). No studies have explored the long term effects of antenatal glucocorticoid exposure on HPAA activity in humans.

A recent study has examined the implications of an unbalanced diet during pregnancy on HPAA activity in young adults. In the late 1960's, at a hospital in Motherwell, UK, pregnant women were advised to eat 1lb of red meat daily and to avoid carbohydrate-rich foods. In a follow up study, offspring of mothers who ate the most meat/fish and the least green vegetables per day in late pregnancy had higher fasting plasma cortisol concentrations (109). Interestingly, they also had higher blood pressures.

1.4.2.2 Epidemiology

Follow up studies similar to those described in section 1.1 have examined the associations between size at birth and HPAA activity. One of the first publications examined data from the Hertfordshire cohort, reporting an inverse relationship between birthweight and fasting morning cortisol concentrations in men aged 60-71 years. Fasting plasma cortisol fell from 408nmol/l in those who weighed 5.5lb (2.49kg) or less to 309nmol/l among those who weighed more than 9.5lb (4.31kg) (110). This trend was paralleled by the concentrations

of 'free cortisol', estimated by the ratio of cortisol to CBG, and was independent of the current obesity. Although a single fasting cortisol measurement has substantial limitations, this observation suggested the possibility of an important link between size at birth and HPAA activity in adult life and generated considerable research interest in the field.

The results of this study have been confirmed in population samples of 165 men and women from Adelaide, South Australia aged 21 years, 199 men and women from Preston, UK aged 45-54 years and 306 women from Hertfordshire, UK aged 60-71 years (111). Low birthweight was associated with raised fasting plasma cortisol concentrations in all three populations and a combined analysis which allowed for differences in the gender composition, age and body mass index (BMI) between the studies showed that cortisol concentrations fell by 23.9 nmol/l per kg increase in birthweight (95%CI, 9.6 to 38.2, p<0.001).

Not all studies have been confirmatory. Clark and colleagues found a U-shaped relationship between urinary cortisol metabolites and birthweight in nine year old children (112). A large cross-sectional study of men and women aged 69 in Finland showed an inverse relationship between birthweight and morning cortisol concentration, but only in those individuals born before 39 weeks gestation. The relationship was reversed in the subjects who had been born after 40 weeks gestation, suggesting heterogeneity in the long term effects of antenatal programming as frequently seen in animal models (113). No association between birthweight and morning cortisol concentration was found in South Asians (114).

Frequent sampling to determine diurnal cortisol rhythm has failed to reproduce the results of studies which have used a single 0900h cortisol concentration as a marker of HPAA activity. Serum cortisol was measured every twenty minutes for 24 hours in eighty-three members (45 men) of the Hertfordshire cohort after a overnight stay in the clinic. Three meals were taken during this time. Thorough analysis of these profiles revealed no influence of birthweight on diurnal cortisol secretion (115). A study comparing small for gestational age children with normally grown controls looked at cortisol concentrations every four hours over a 24 hour period, after acclimatisation to the hospital environment for at least a day prior to sampling. There were no differences in cortisol between the two groups and no associations with size at birth in continuous analysis (116). These results raise the possibility that it may be altered stress responsiveness that is programmed antenatally in humans, as a fasting morning cortisol concentration taken in a novel clinic setting may better reflect the reactivity of the HPAA than its resting status.

A more detailed assessment of HPAA function was carried out on a subset of 203 men from the original Hertfordshire study. They underwent an overnight low dose (0.25mg) dexamethasone suppression test (DST) followed by a $1 \mu g$ ACTH$_{1-24}$ stimulation test. A 24h urine sample was collected for analysis of cortisol metabolites by gas chromatography/electron impact mass spectrometry. Men with lower birthweight had enhanced responses of plasma cortisol to ACTH$_{1-24}$ (p=0.03) and increased total urinary cortisol metabolite excretion (after adjustment for confounding effects of increased obesity and lean body mass in high birthweight men), but did not differ in post-dexamethasone cortisol concentration (117). The protocol was also performed in women and similar results have been reported recently. In a combined analysis adjusted for gender, a 1lb decrease in birthweight was associated with a 13.4nmol/l (CI: 4.5 to 22.4, p<0.003)

increase in peak plasma cortisol after ACTH stimulation (118). A study in 20 year old

South Africans has supported these observations, showing higher fasting cortisol and

exaggerated ACTH responsiveness in those who were underweight at birth (119).

Together, these studies provide a reasonable body of human data to support the hypothesis

that HPAA hyperactivity may be programmed in utero in response to environmental

factors that result in lower birthweight.

1.4.2.3 Relevance to the metabolic syndrome

To investigate the possibility that programmed alterations in HPAA activity may be an

intermediary mechanism in the fetal origins of adult disease, several of the above studies

have also looked for associations between cortisol and cardiovascular risk factors. In 64

year old men, 0900h cortisol concentrations were positively correlated with systolic and

diastolic blood pressure, fasting and two hour glucose, insulin resistance (defined by the

homeostasis model assessment) and triglyceride concentrations (110). These relationships

generally added to the effect of obesity in predicting cardiovascular risk, but interactions

between cortisol concentration and obesity (BMI or waist hip ratio (WHR)) have been

found in some analyses. For example, the relationship between cortisol and blood pressure

was strongest in those individuals with the highest BMI (111). A higher peak plasma

cortisol after ACTH stimulation was also associated with cardiovascular risk factors and,

in multiple logistic regression analysis, birthweight, peak plasma cortisol and BMI were

all independent predictors of the metabolic syndrome (117).

1.5 THE SYMPATHOADRENAL SYSTEM

1.5.1 Overview

Alongside the HPAA, the sympathoadrenal system is the other major effector of the stress response. Centrally perceived stress increases noradrenergic output from the brainstem, principally the locus coeruleus. From here, neurones project throughout central nervous system. The sympathetic response is produced through activation of the sympathetic nervous system and the adrenal medulla. The sympathetic nervous system comprises an array of neurones originating in the thoracolumbar spinal cord which, after synapsing within the sympathetic ganglia, supply virtually all tissues. The main neurotransmitter at post-ganglionic nerve terminals is noradrenaline. In the adrenal medulla, pre-ganglionic nerve fibres stimulate chromaffin cells to secrete adrenaline. At the cell surface, these two hormones, the catecholamines, interact with α and β-adrenoreceptors. The sympathoadrenal response is typified by the classic 'fright or flight reaction': papillary dilatation, increased heart rate and contractility (increased cardiac output), bronchiolar dilatation (increased ventilation), redistribution of peripheral blood flow to skeletal muscle from skin and viscera, and enhanced glycogenolysis (mobilisation of fuel).

1.5.2 Programming of the sympathoadrenal system

There is considerably less information in the literature about early life influences on the activity of the sympathoadrenal system. This is partly due to methodological difficulties; each tissue needs to examined independently to determine differences in sympathetic innervation and measurement of circulating catecholamine concentrations provides only a crude index of sympathetic activity.

Thermoregulation is one area that has been investigated in some detail. Studies describing the differential development of sweat glands in humans were outlined in section 1.2. In rats, a similar phenomenon has been described whereby animals reared in cold temperatures are better able to withstand cold later in life and *vice versa*. This has recently been shown to be due to differential sympathetic innervation (assessed by noradrenaline turnover) of brown adipose tissue, the main heat-generating tissue in rodents (120). The same group have investigated the effect of neonatal handling on sympathoadrenal activity. They found evidence of reduced sympathetic innervation in spleen and heart, no alterations in brown adipose tissue, kidney and stomach and an increased adrenaline response to fasting (121).

Studies in rodent offspring have shown reduced noradrenaline content in specific tissues following various prenatal exposures, including dexamethasone administration, maternal diabetes, ethanol and nicotine exposure, and protein-restriction. The long term effects of these alterations are unknown (122). Prenatal influences on circulating catecholamines have also been assessed in some studies. Protein restriction (50%) through pregnancy and lactation in rats resulted in increased circulating adrenaline and noradrenaline concentrations in male offspring at three months of age and also increased the expression of β_1 and β_3 receptors (123). However, another group found no differences in catecholamine concentrations when maternal food intake was limited globally to 50% normal (85). Using uterine artery ligation to induce fetal growth retardation, Jannson and colleagues found that an index of total body sympathetic activity and plasma catecholamine concentrations were inversely related to birthweight in the female offspring (40). Unpredictable noise and light stress during gestation did not alter resting catecholamine concentrations, but induced a greater rise in noradrenaline concentration in

response to novelty and footshock than in control rats at five months of age (124). Prenatal dexamethasone exposure was associated with increased resting plasma catecholamine concentrations in male rats, though due to the small numbers in this experiment the results did not reach statistical significance (125).

There is a reasonable body of work in sheep examining the development of the sympathoadrenal system in late fetal life under different environmental conditions. In one model, placental restriction from conception (carunclectomy) increased circulating catecholamine concentrations in late gestation, but impaired the adrenaline response to subsequent hypoxia. This was associated with reduced adrenaline synthetic capacity of the fetal adrenal medulla, but greater release of noradrenaline following tyramine infusion (126). This work has led to the suggestion that chronic hypoxia in utero may be a stimulus to sympathetic hyperinnervation (127). There are no postnatal studies in the model.

Human data examining early life influences on sympathoadrenal function are scanty. It is well recognised that growth-retarded fetuses are often tachycardic and have raised catecholamine concentrations in cord blood (128). In one study, small-for-gestational age new-borns had increased heart rate and reduced heart rate variability when compared with controls during sleep (129). Resting pulse rate was inversely related to birthweight in 266 men and women aged 50 (decline in pulse rate per kg increase in birthweight: 2.7 beats/min, 95% CI 0.6 - 4.8) (130). This finding was independent of gestational age, adult BMI or WHR and remained after excluding those individuals with known cardiovascular disease. Similar results were seen in 2648 African school children (131). Inverse associations between resting pulse rate and birthweight suggest the possibility of autonomic nervous system programming, though clearly this data needs to be backed up

with more specific testing (130, 131). A recent case control study found that twelve year

old children born small for gestational age had elevated circulating adrenaline, but similar

noradrenaline concentrations (132).

In summary, these preliminary studies provide some support for the hypothesis that

sympathoadrenal activity may be programmed by events in early life, with potential long

term influences on cardiovascular function.

1.6 INTERACTIONS BETWEEN THE HPAA AND THE SYMPATHOADRENAL SYSTEM

The two arms of the stress system are closely linked anatomically and functionally, both centrally and in the periphery. The degree to which one system regulates the other has been the subject of some controversy, largely due to contradictory publications. A full review of the literature on this subject is beyond the scope of this introduction. However, there is reasonable evidence of reciprocal stimulation of the two axes centrally; stress-induced rises in noradrenaline increase CRH expression in the PVN and CRH stimulates noradrenaline release from the locus coeruleus (133, 134).

Endogenous glucocorticoids appear to restrain neuronal catecholamine synthesis, turnover, release, reuptake and metabolism under basal conditions and following stress, and circulating catecholamine concentrations are therefore elevated following adrenalectomy. A single bolus of cortisol suppresses sympathoadrenal activity, but chronic administration activates the system showing that interactions between the axes are dynamic (135).

Within the adrenal gland, there is also interaction between the two systems. Phenylethanolamine N-methyl transferase (PNMT), the final enzyme in the adrenaline biosynthetic pathway, has a glucocorticoid sensitive gene and dexamethasone administration has been shown to upregulate PNMT mRNA. Hypophysectomy reduces adrenal PNMT, and this can be restored by treatment with ACTH (135). Recent data has shown that the rate-limiting step in cortisol production, transport of cholesterol across the mitochondrial membrane of adrenocortical cells, is enhanced in the presence of chromaffin cells or by including adrenaline in the culture medium. This occurs via enhanced transcription of steroidogenic acute regulatory protein (136).

1.7 SUMMARY AND OBJECTIVES

Epidemiological studies have suggested that fetal growth restriction may have long term effects on health. These observations have opened up a novel approach to understanding the pathophysiology of chronic diseases such as ischaemic heart disease and diabetes. The 'fetal origins' hypothesis proposes that adverse environmental exposures, for example undernutrition, during critical periods of development result in programmed alterations in physiology and structure that may be disadvantageous to the individual in later life.

Research is now focusing on the mechanisms that may underlie this hypothesis. The central role of glucocorticoids in many physiological processes and studies linking the HPAA to conditions such as the metabolic syndrome make this hormonal system a likely candidate. In animal models, various antenatal manipulations have been shown to alter HPAA activity in the offspring and recent epidemiological studies in humans have provided evidence of inverse associations between size at birth and morning cortisol concentrations and adrenal responses to ACTH.

The nature of the HPAA abnormality that results from fetal growth restriction in humans is not yet understood. Circulating plasma cortisol concentrations depend on the relative rates of production and metabolism of cortisol. The research in this thesis was undertaken to examine the hypothesis that cortisol production may be upregulated by central mechanisms in low birthweight individuals. Two separate studies were performed.

Firstly, responses to CRH were compared in a group of low and high birthweight men from a new cohort in Hertfordshire, UK. The test was carried out on two occasions, the second after an overnight dexamethasone suppression test, to examine the effect of size at

birth on corticotroph responsiveness, central drive to the corticotroph and feedback control of the HPAA. Background data was available on the participants allowing an assessment of the relationship between these measures of HPAA activity and features of the metabolic syndrome.

The second study was performed in a large group of young adults from an established birth cohort in Adelaide, South Australia. Salivary cortisol concentration and blood pressure were measured while the participants undertook a series of psychological stress tests to assess the influence of size at birth on adrenocortical and haemodynamic responses to stress.

CHAPTER 2

Central regulation of the pituitary-adrenal axis: relationships with fetal growth and the metabolic syndrome.

2.1 BACKGROUND

The experimental approach used in this study was drawn from the literature on major

depression. Researchers in the field of biological psychiatry have been interested in the

HPAA since hypercortisolism was first reported in patients with depression over forty

years ago (137). Abnormal HPAA activity has been repeatedly described in depressed

patients, in particular elevated urinary free cortisol excretion, abnormal diurnal rhythms of

cortisol secretion, increased ACTH responsiveness and reduced dexamethasone

suppressibility (138-140).

Since the isolation of CRH in 1981 (141), considerable work has gone into understanding

the neuroendocrine mechanisms behind this enhanced pituitary-adrenal activity, leading to

the hypothesis that overactive CRH neuronal circuits may be the pathophysiological basis

for depression (142). Nemeroff and co-workers have shown that CRH concentrations are

elevated in the cerebrospinal fluid (CSF) of depressed patients and this group have also

reported reduced CRH receptor binding in the prefrontal cortex of depressed suicide

victims (143, 144). This data has been challenged (145), but one remarkably consistent

finding is that the ACTH response to CRH is blunted in patients with major depression

compared with normal controls (146, 147). This pattern is the opposite to that seen in

hypercortisolaemic patients with Cushing's disease who have a large ACTH response

during the CRH test. In depression, it is thought that the corticotroph is being

appropriately constrained by high circulating cortisol concentrations and, therefore, that

the HPAA abnormality is at the level of the hypothalamus or above, resulting in

hypersecretion of CRH (147). This hypothesis has been supported by a study which found

increased CRH mRNA expression in the PVN of depressed patients in post mortem

studies (148).

In the late 1980's, Holsboer and colleagues suggested a novel means of investigating

subtle HPAA dysfunction: performing a CRH test after suppressing the axis with

dexamethasone (DEX/CRH test) (149, 150). It has since been shown that the DEX/CRH

test correlates more closely with measures of diurnal HPAA activity than the standard

DST in both healthy and depressed individuals (151). Depressed patients tend to show

enhanced cortisol responses compared with controls in the DEX/CRH test (152).

One explanation for this finding is that the DEX/CRH test is a marker of vasopressinergic

drive to the pituitary. Dexamethasone, a synthetic steroid, does not freely cross the blood

brain barrier and the majority of its suppressive action is at the level of the pituitary (153).

It reduces endogenous glucocorticoid production which may result in a state of relative

glucocorticoid depletion within the brain and a reduction of negative feedback. In

depression, where an increased proportion of CRH-containing neurones in the PVN co-

express AVP (148), this leads to increased release of AVP into the hypophysial portal

circulation which acts synergistically with exogenous CRH to overcome dexamethasone

suppression at the pituitary (152).

This hypothesis has been backed up by a recent animal study in which the responses to a

DEX/CRH test were compared in two lines of Wistar rat: high anxiety and low anxiety.

The high anxiety rats showed exaggerated ACTH and corticosterone release which was

associated with increased basal AVP expression and release in the PVN and was blocked

by a selective vasopressin-1 receptor antagonist (154). Other researchers have suggested that the DEX/CRH test is a means of unmasking subtle defects in feedback regulation (155, 156).

Basal adrenocortical activity is often assessed using a 0900h blood sample which is assumed to measure the diurnal cortisol peak. However, the normal range for morning cortisol concentration is large, overlapping with pathology (hypoadrenalism and Cushing's syndrome) at both ends, and it is not a particularly reproducible measure within individuals (r = ~0.5) (157, 158). There is evidence that HPAA activity peaks shortly after waking and it follows that measuring cortisol at a specific clock time will not be reliable as a means of catching the peak (159).

Unbound cortisol passes freely into saliva by diffusing through the cells of the salivary gland and its concentration is therefore independent of saliva flow rate (160). It is possible to accurately determine salivary cortisol concentration with modern assay techniques and salivary cortisol has been shown to closely mirror fluctuations in total plasma cortisol (161). Saliva collection is simple and non-invasive, allowing measurements to be made outside the laboratory setting and avoiding stress-induced rises in cortisol concentration that may be induced by venepuncture.

The salivary cortisol response to awakening has been proposed as a useful measure of HPAA activity (162). Pruessner and colleagues have shown that the rise in salivary cortisol concentration during the first hour after waking is stable within individuals over a number of weeks (162). They have demonstrated exaggerated rises in subjects exposed to chronic stress associated with work overload, possibly representing a stress response in

anticipation of the demands of the day ahead, and reduced responses in teachers with high burnout scores (163, 164). In a recent study, overall cortisol production in the first 45 minutes after waking was shown to correlate with 12-hour diurnal mean cortisol (sampled 3 hourly from waking) while the response to awakening was not, suggesting that this measure may be a dynamic test of HPAA function (165)

The features of pituitary-adrenal overactivity seen in low birthweight individuals, namely increased fasting 0900h cortisol concentration and adrenal responsiveness to ACTH (as detailed in section 1.4.2), mirror those found in depressed patients suggesting that reduced fetal growth might also be associated with increased CRH or AVP drive to the pituitary-adrenal axis. To examine this hypothesis, the responses to awakening, CRH and DEX/CRH tests were compared in a group of low and high birthweight men recruited from a birth cohort in Hertfordshire, UK. Blood pressure and metabolic data on these individuals had previously been collected allowing an assessment of the relationship between central HPAA regulation and cardiovascular risk factors.

2.2 METHODS

2.2.1 Participants

2.2.1.1 The Hertfordshire cohorts

From 1911 to 1948 detailed infant records were collected in the county of Hertfordshire, UK. Birthweight was recorded by the attending midwife and health visitors documented how the baby was fed, weight at 12 months and any infections experienced in the first year. These records were collated centrally into large ledgers which were discovered in the archive at Hertford County Hall in the mid 1980s. Individuals born and still living in Hertfordshire have been traced by the MRC Environmental Epidemiology Unit in Southampton with the aid of the NHS central registry in Southport. Early data in support of the 'fetal origins' hypothesis was collected from a cohort of individuals born between 1911 and 1930 (see section 1.1). This group are hereafter referred to as the older Hertfordshire cohort.

In 1998, recruitment of a new Hertfordshire cohort commenced to examine further the early origins of coronary heart disease, type 2 diabetes and osteoporosis, in particular the interactions between early life, adult diet and lifestyle, and genetics. The aim was to establish a group of 5000 men and women born countywide between 1931 and 1939. 12,250 men from singleton pregnancies with complete birthweight information recorded in the ledgers were identified, of whom 1760 were traced as currently living in the East Hertfordshire region. Recruitment for the new cohort began with these men and the study samples for the work in this chapter were drawn from this population. With the approval of their General Practitioners, 1397 men were contacted by post to advise them of the study and 768 (55%) agreed to take part, forming the new East Hertfordshire cohort.

The first contact was at the individual's home where a trained research nurse obtained detailed information about their medical and social history, socioeconomic status, exercise patterns, smoking and alcohol consumption by means of an administered questionnaire (Appendix A). Mood was assessed with the hospital anxiety and depression (HAD) score (166).

2.2.1.2 Baseline assessment

The participants attended a clinic at Hertford County Hospital after an overnight fast for baseline anthropometric, haemodynamic and biochemical assessment. Height was measured to the nearest 0.1cm using a Harpenden pocket stadiometer (Chasmors Ltd, London, UK) and weight to the nearest 0.1kg on a SECA floor scale (Chasmors Ltd, London, UK). BMI (weight divided by height^2) was calculated. Waist (mid way between the costal margin and the iliac crest in the mid axillary line) and hip (greatest diameter around the gluteal region) circumferences were measured with a steel tape and skinfold thickness was determined at four sites (biceps, triceps, subscapular and suprailiac) using Harpenden skinfold callipers (Chasmors Ltd, London, UK). WHR was calculated and total body fat (%) was derived from the skinfolds according to the equations of Durnin and Womersley (167). Subscapular to triceps skinfold ratio (SSTR) was calculated as a measure of truncal fat.

Fasting blood samples were withdrawn for glucose, lipid profile, insulin and precursors, and cortisol and then a standard 75g OGTT was performed, sampling at 30 and 120 minutes for glucose and insulin. Blood pressure was measured with a Dinamap Model 8101 (GE Medical Systems, Slough, UK) after being seated for at least five minutes; a mean of three recordings was taken. An assessment of inter-observer variability was made

every six months to ensure the accuracy of these measurements. This work was undertaken by the Hertfordshire 31-39 Study Group and the information collected has kindly been made available for use in this thesis.

2.2.1.3   HPAA study

A sample of men from the top and bottom quartiles of birthweight were recruited for the HPAA study. A factorial design was decided upon to increase the power of the study given the time and expense involved in CRH testing. Studies of patients with major depression have revealed up to 50% suppression of the ACTH response to CRH compared with normal controls (147, 168). For this study, as differences in basal cortisol secretion between birthweight groups are less marked than in depression, 30% suppression was felt to be a plausible difference. A sample size of 102 was necessary to detect a 30% difference in the ACTH response to CRH between the groups with 80% power at the 5% significance level (based on a mean ACTH response (incremental area under the curve) of 115 pmol/min.L (SD 63) (169)). The 25th (7lbs) and 75th (8.5lbs) percentiles for birthweight in the population were determined from the ledger data of all the men who had been traced. At the time of sampling for the HPAA study, 597 men in the East Hertfordshire region had completed the baseline assessment; 145 in the bottom and 139 in the top quartile of birthweight. Permission was once again sought from General Practitioners and individuals were excluded if they had a history of pituitary or adrenal disease, diabetes or either major depression or glucocorticoid treatment in the previous three months. 122 men (59.5% of those suitable) were recruited; 58 in the low birthweight (LBW) group and 64 in the high birthweight (HBW) group (see Figure 2.1). Ethical approval was sought from the North and East Hertfordshire Local Research Ethics Committee and all participants gave written informed consent.

Figure 2.1 Recruitment of participants for the HPAA study.
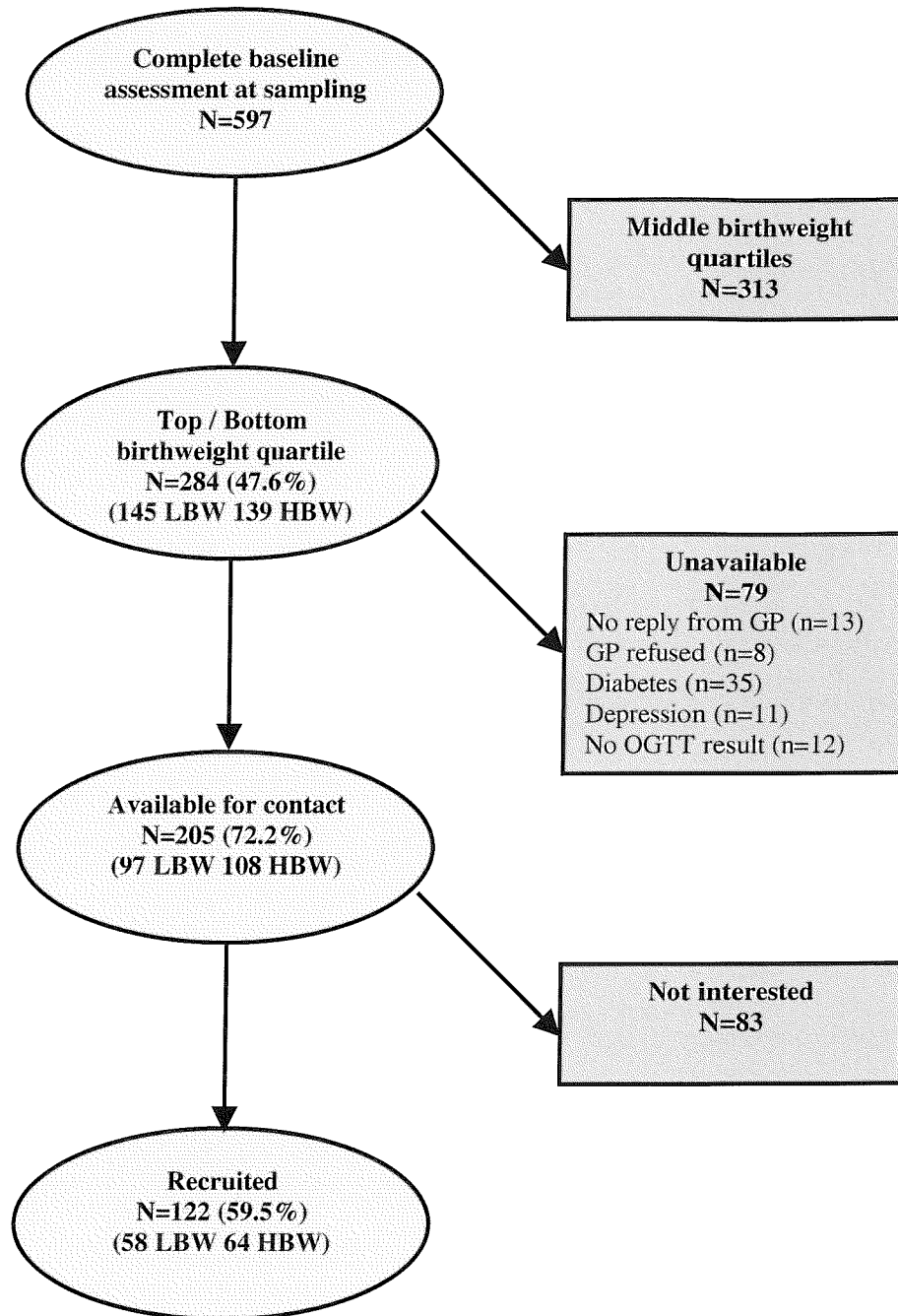
LBW: Low birthweight (<7lbs), HBW: High birthweight (>8.5lbs)
OGTT: Oral glucose tolerance test

2.2.2   Salivary cortisol response to awakening

After agreeing to take part in the study, participants were sent five Salivettes (Sarstedt, Leicester, UK) with which to collect saliva. The night before their clinic appointment, they were asked to fast from midnight and then to collect a saliva sample at 0, 15, 30, 45 and 60 minutes after waking the following morning. They remained fasted during this period and were asked to refrain from brushing their teeth to prevent contamination of the saliva samples with blood. They brought the samples with them to the clinic.

2.2.3   CRH test

Intravenous administration of CRH results in a dose-dependent rise in ACTH and cortisol (170). Both human and ovine CRH are available for experimental use, differing in only seven amino acids. Ovine CRH produces a biphasic ACTH response due to a biexponentional decay curve, whereas human CRH produces a single peak (171). CRH tends to be administered in one of two doses by different research groups, either 1μg/kg or 100μg regardless of body weight (147, 172). The former regimen is standard practice in the United States, whereas the latter tends to be used by European groups. On the advice of Professor Ashley Grossman (St Bartholomew's Hospital, London), 100μg human CRH was chosen for the present study. This is also the dose used for the DEX/CRH test (see below) allowing continuity between the two arms of the study.

Participants attended the clinic at 0800h on the morning of their saliva collection. A 21-gauge canula was inserted into an antecubital vein and the subject then rested for 30 minutes in a semi-recumbent position. Baseline blood samples were drawn at −15 and −5 minutes before CRH administration. At 0900h, 100μg human CRH (Ferring Pharmaceuticals, Slough, UK) was injected as a bolus and flushed through with 10mls

0.9% saline. Lyophilised CRH was reconstituted in the supplied diluent immediately prior to administration. 10ml blood samples were then drawn from the canula at 5, 15, 30, 45, 60, 90 and 120 minutes. The patency of the canula was maintained with regular saline flushes. Blood for ACTH analysis was collected into chilled EDTA tubes, stored on ice, spun at 4°C within 20 minutes and the plasma was immediately frozen to –80°C until assayed. Serum for cortisol analysis was prepared from clotted samples. After removal of the canula, the participants were given breakfast.

CRH administration resulted in facial flushing in 77% of the subjects. Five men experienced a brief vasovagal episode within 10 minutes of CRH injection and two men became unwell during the course of the study (one felt dizzy and the other developed epigastric pain, a symptom for which he was under investigation). Examination of the ACTH and cortisol response profiles in these subjects showed extremely high values compared with other participants and they were therefore excluded from further analysis. So too were the data from a needle phobic subject who had very high baseline values and failed to respond to CRH. One subject's baseline blood samples were accidentally frozen rendering them unsuitable for ACTH analysis. Thus, complete ACTH and cortisol data were available for 113 and 114 men respectively.

2.2.4 DEX/CRH test

One hundred and fifteen men who had completed the CRH test without side effects were invited to attend on a second occasion for the DEX/CRH test. This visit was at least a month after the first to ensure that the results were not influenced by the first test (mean interval 9 weeks (range 5-21). 103 (46 LBW) agreed to take part. The participants were given 1.5mg dexamethasone to take at 2300h on the night before the study. They were

asked to abstain from alcohol on that evening and to have breakfast as usual the following morning but to avoid caffeine-containing drinks. They attended the clinic at 1230h on the study day and were given a standard sandwich lunch to ensure that all subjects had equivalent calorie and electrolyte intake before the DEX/CRH test. At 1330h, a canula was inserted into an antecubital vein and the subject then rested in a semi-recumbent position for 30 minutes. Baseline blood samples were withdrawn at −15 and −5 minutes prior to injection of 100μg human CRH. Following the injection, blood was sampled at 15, 30, 45, 60, 75, 90, 105 and 120 minutes for ACTH and cortisol. Sample preparation was as described in section 2.2.3. None of the men experienced any adverse effects during the DEX/CRH test.

2.2.5   Laboratory assays

All assays were performed under the supervision of Dr Peter Wood in the Regional Endocrine Laboratory at Southampton General Hospital. Salivary cortisol was measured using a time-resolved fluorescent immunoassay ("DELFIA" system). Examination of the inter-assay precision profile for the salivary cortisol assay showed that the coefficient of variation (CV) was 5–10% between 2 and 15 nmol/l cortisol. The assay had a lower limit of detection of 0.4 nmol/l. The cross-reactivity of cortisone in the salivary cortisol assay was 2.5%. As the mean cortisol:cortisone ratio at this time of day is between 0.32 and 0.48 (173), the contribution of cortisone to the cortisol results was less than 1 nmol/L. Serum cortisol was assayed using an in-house radioimmunoassay (174). An extra low standard (15.6 nmol/L) was included to give additional resolution at low concentrations. The inter-assay CV at this concentration was 14.9% and ranged from 7.4 − 10.3% within the normal range of cortisol concentrations. Plasma ACTH was measured with a highly sensitive commercial assay (Nichols Institute, CA) to give precise results at low ACTH

concentrations. The detection limit of this method is 0.2 pmol/l, approximately ten times lower than most conventional ACTH methods. The inter-assay CV at a concentration of 1.1 pmol/l was 10.4% and ranged from 6.8 – 7.8% between 7.9 and 78.8 pmol/l ACTH.

Eighteen months after the analysis of the DEX/CRH test was completed, an error was discovered; the ACTH assays had been performed on the serum samples rather than the plasma samples that had been carefully prepared to prevent degradation of ACTH. As repeating all the assays would have involved considerable time and expense, the plasma samples were analysed for ten subjects (ie 50 samples) and these results were compared with those from the serum samples. The results were closely correlated (r=0.92 (95% CI 0.86 to 0.95), p<0.0001), but the ACTH concentrations in the plasma samples were consistently higher confirming that degradation of the hormone had indeed occurred prior to freezing the serum samples (Figure 2.2). The difference between the values was not biased by the time at which they were taken in the study. It was decided that the overall results of the study would be unlikely to be influenced by this mistake, but that the descriptive statistics should be scaled by the slope of the regression equation to allow better comparison with the literature. Hence, all the ACTH results for the DEX/CRH test presented in this chapter are serum ACTH concentration (pmol/l) multiplied by 1.884. The statistical analysis was performed on the untransformed data.

Figure 2.2 Comparison of DEX/CRH test serum and plasma ACTH concentrations in 10 subjects.
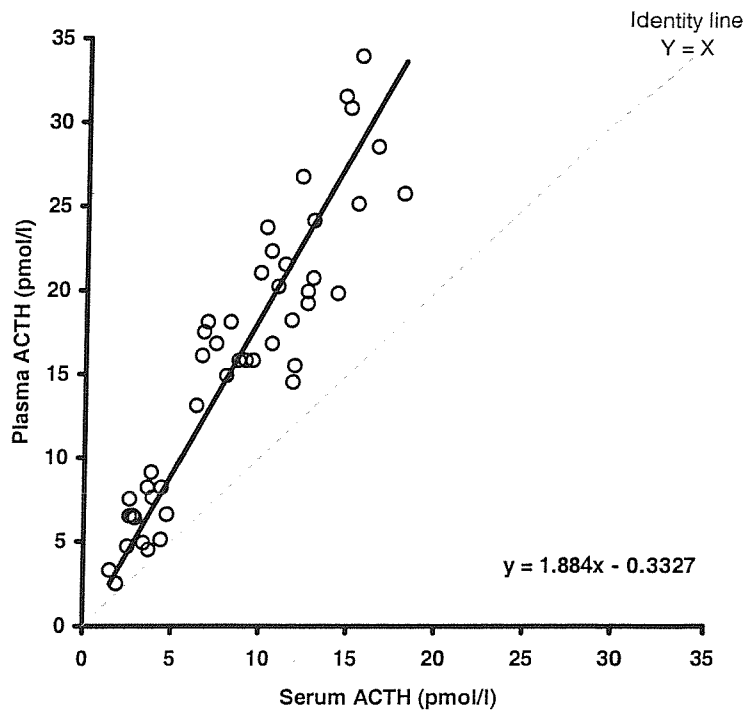
Intact insulin, proinsulin and 32,33 split proinsulin were measured by in-house immunofluorimetric two-site assays ("DELFIA" system, Perkin Elmer Ltd, Milton Keynes) using monoclonal antibodies purchased from Dako Ltd (Ely, Cambs). The methods were based on those described by Sobey and colleagues (175). Inter-assay imprecision (CV) ranged from 6 - 10 % for low, medium and high quality control samples for intact insulin. Proinsulin cross-reacted 100% in the proinsulin + 32,33 split proinsulin assay and therefore 32,33 split proinsulin results were obtained by subtraction of the specific proinsulin assay results from those for proinsulin + split proinsulin. Inter-assay imprecision ranged from 7 - 15 % for low, medium and high quality control samples. Glucose and lipids were assayed on an Advia 1650 autoanalyser (Bayer diagnostics, UK).

2.2.6 Statistical analysis

Diabetes was defined according to current WHO criteria (fasting plasma glucose ≥ 7.0 or 2hr plasma glucose ≥ 11.1) (176). Men with impaired glucose tolerance (2hr glucose 7.8-11.0) or type 2 diabetes, a systolic blood pressure >160mmHg or antihypertensive treatment and a fasting plasma triglyceride concentration above the median (1.4mmol/l) were defined as having the metabolic syndrome. Insulin resistance (HOMAr = $(0.149*(ins0+proins+split))/(22.5*(exp(-1*(ln(gluc0))))))$ and insulin secretion (HOMAβ = $(2.98*(ins0+proins+split))/(gluc0-3.5))$ were estimated using the homeostasis model assessment (177).

Fasting 0900h cortisol concentration in the HPAA study was determined from the mean of the pre-CRH test samples. The mean of the two baseline samples in the DEX/CRH test represented an overnight 1.5mg DST. The cortisol response to awakening and the ACTH and cortisol responses in the two CRH tests were assessed by a number of parameters:

1. Peak: the maximum value following stimulation

2. Increment: peak minus baseline

3. Percentage increase from baseline to peak

4. Area under the curve (AUC) calculated by the trapezoidal rule

5. Incremental AUC (iAUC = AUC minus baseline area)

6. Time of peak

In all three tests, the iAUC proved the most useful summary measure of the response and thus, along with most published studies, the descriptive statistics and associations presented in this chapter focus on this variable.

Log_e-transformation of skewed variables was performed where necessary and corresponding geometric means and standard deviations (sd) are presented. ACTH iAUC in the CRH test and cortisol and ACTH iAUC in the DEX/CRH test were transformed to normality using Fisher-Yates normal scores as negative values prevented logarithmic transformation. T-tests and regressions were based on these variables but descriptive statistics (median and interquartile range (IQR)) are presented on the untransformed scale for simplicity.

Baseline and stimulated pituitary-adrenal activity in the LBW and HBW groups were compared using unpaired t-tests. The possible confounding effects of age, obesity, smoking and alcohol consumption, social class, exercise patterns and marital status (adult lifestyle variables) were examined using multiple linear regression. The ACTH and cortisol response profiles were also analysed longitudinally. The longitudinal approach considers the full series of data for each subject and uses a generalised estimating equation to model the average response during the test in relation to the factors of interest, taking into account the effects of time and the autocorrelation of measurements within each subject (178).

Associations between baseline and stimulated pituitary-adrenal activity and the components of metabolic syndrome were assessed using Pearson correlation coefficients, correcting for the possible confounding effects of age, obesity and adult lifestyle variables in multiple linear regression models. Multiple logistic regression was used to explore the binary response variables defined above. All analyses were performed using Stata Statistical Software Release 7.0 (Stata Corporation, College Station, TX).

2.3 RESULTS

2.3.1 Characteristics of the participants

At the end of recruitment in the East Hertfordshire region, 768 men had agreed to join the new cohort of whom 678 (88%) had complete data from the baseline assessment clinic, including a fasting 0900h plasma cortisol concentration. Part of the analysis in this chapter is based on these 678 men. They were aged between 59 and 70 years (mean 64.3yrs) and had a mean birthweight of 3.53kg (sd 0.55) (7.8lbs (1.2)). Birthweight was 0.06kg (0.1lb) higher in these men than in the remainder of those traced in the East Hertfordshire region (n=1082, p=0.03). A summary of their anthropometry, adult lifestyle factors (smoking status, alcohol consumption, physical activity, social class and marital status) and depression score is given in Table 2.1.

One hundred and twenty-two men were recruited for the HPAA study (see section 2.2.1.3 and Figure 2.1). The characteristics of these participants are also detailed in Table 2.1. There were no significant differences in anthropometry, adult lifestyle factors or depression score between those who took part in the HPAA study and those who did not. 58 men formed the LBW group (mean birthweight 2.8kg (range: 2.0 to 3.1) (6.2lb (range: 4.5 to 6.9))) and 64 the HBW group (4.3kg (range: 4.0 to 5.0) (9.5lb (range: 8.8 to 11.0))). The subjects were aged between 60.5 and 69.6 years in both birthweight groups. The LBW group were significantly shorter and lighter as adults, but there were no other significant differences between the two groups, apart from marital status which reflected in part the proportion of men who had never married in the cohort as a whole; LBW 4.8% HBW 8.4%. None of the subjects were suffering from depression (HAD score >10). The characteristics of the 103 men who completed the DEX/CRH test did not differ from the 122 initially recruited for the HPAA study (data not shown).

Table 2.1   Characteristics of men in the East Hertfordshire cohort with a complete baseline assessment and of the HPAA study group, as a whole and in the two birthweight groups.

	Whole cohort	HPAA study	LBW	HBW
N	678	122	58	64
Age (y)	64.3 (2.6)	64.1 (2.7)	64.1 (2.7)	64.1 (2.9)
Birthweight (kg)	3.53 (0.55)	3.58 (0.79)	2.80 (0.23)	4.29 (0.26)[b]
Height (m)	1.74 (0.07)	1.74 (0.07)	1.72 (0.06)	1.76 (0.08)[b]
Weight (kg)[a]	80.8 (1.2)	79.7 (1.1)	76.7 (1.1)	82.3 (1.1)[b]
BMI (kg/m^2)[a]	26.7 (1.1)	26.4 (1.1)	26.0 (1.1)	26.8 (1.1)
WHR	0.96 (0.06)	0.95 (0.06)	0.95 (0.05)	0.96 (0.06)
Body fat (%)	28.7 (5.3)	27.9 (5.1)	28.2 (4.1)	27.6 (6.0)
SSTR[a]	1.71 (1.4)	1.65 (1.4)	1.68 (1.4)	1.63 (1.4)
Current smoker (%)	17.1	18.0	15.5	20.3
Alcohol (units/week)[c]	10 (3-23)	10 (4-25)	9 (4-21)	13 (4-27)
Activity score[c]	64 (57-71)	64 (50-71)	64 (50-71)	64 (57-75)
Social class I-IIINM (%)[d]	36.7	38.5	39.7	37.5
Never married (%)	5.3	8.2	1.7	14.1[b]
HAD score[c]	1 (0-3)	1 (0-3)	1 (0-3)	1 (0.5-2)

Data are mean (sd). [a]Geometric mean (sd)
[b]HBW > LBW (p<0.01)
[c]Median (interquartile range)
[d]Upper half socioeconomic status, based on 6 tiers: I, II, III non-manual, III manual, IV, V
LBW: low birthweight, HBW: high birthweight
BMI: body mass index, WHR: waist to hip ratio, SSTR: subscapular to triceps ratio
HAD: hospital anxiety and depression scale

2.3.2 Relationship of HPAA activity to potential confounders

2.3.2.1 Baseline assessment

Mean fasting 0900h cortisol concentration at baseline assessment was 339nmol/l (gsd 1.4). Fasting cortisol was positively correlated with age (r=0.10, p=0.01) and inversely associated with obesity measures, most strongly with BMI (r=-0.11, p=0.003) (Table 2.2). 0900h cortisol concentration was higher in those men who drank 10 units of alcohol or more per week than those with minimal alcohol intake (350 vs 330 nmol/l, p=0.01), but it was not related to smoking (non smokers: 340 nmol/l, smokers: 331 nmol/l, p=0.4), social class (I-IIINM: 343 nmol/l, IIIM-V: 337 nmol/l, p=0.5), physical activity (rho=-0.01, p=0.8) or depression score (rho=0.02, p=0.5). Subjects who had never been married had a higher fasting cortisol (394 vs 336nmol/l, p=0.003).

2.3.2.2 Salivary cortisol response to awakening

Salivary cortisol concentrations rose in 82% subjects over the first hour after waking, peaking on average at 30 minutes (Figure 2.3). The mean increase was 10.2 (sd 8.3) nmol/l. Mean total integrated salivary cortisol secretion (AUC) was 1060 (sd 375) nmol/l.min and the mean salivary cortisol response to awakening (iAUC) was 282 (sd 420) nmol/l.min. None of the variables derived from the salivary cortisol profiles were significantly correlated with 0900h cortisol concentration on the morning of the study (r=0.00 - 0.08) and they did not vary with time of waking, age, adult lifestyle factors or depression score. The AUC was not related to any of the obesity measures, but there was a trend towards a lower iAUC with increasing obesity (Table 2.2).

Figure 2.3 Salivary cortisol response to awakening in 122 men aged 65yrs. Data are geometric mean ± sem.
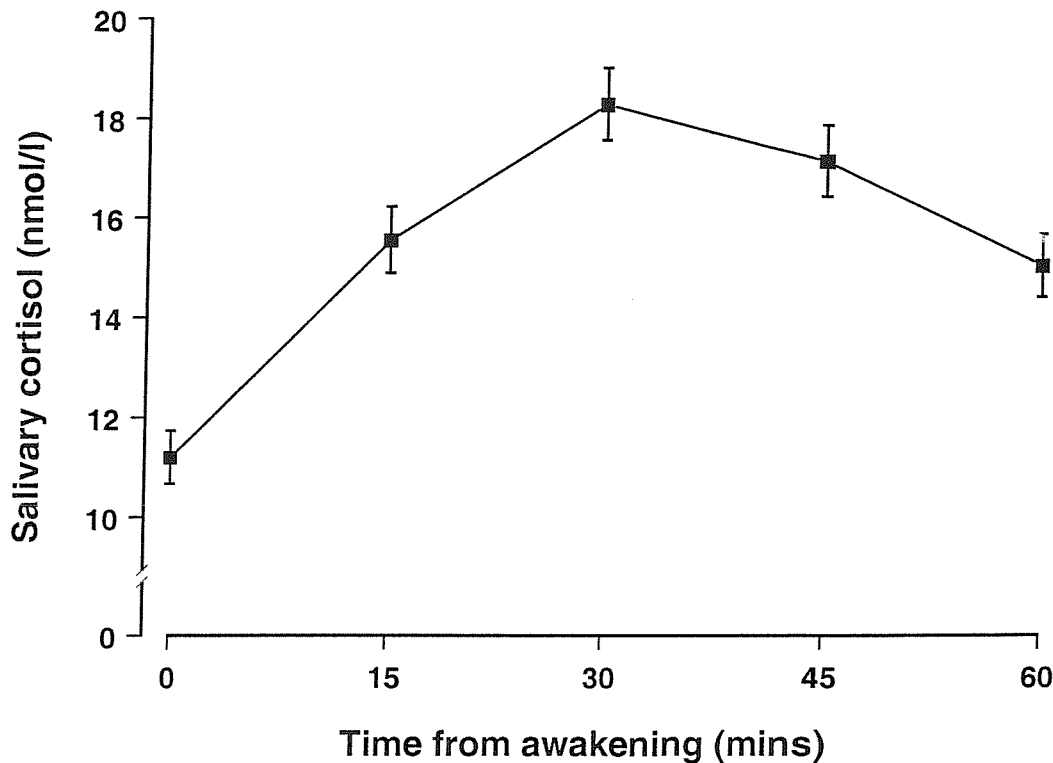
2.3.2.3 CRH test

Mean fasting 0900h serum cortisol concentration was 304 (gsd 1.3) nmol/l prior to the CRH test. This was positively correlated with baseline assessment cortisol (r=0.56, p<0.001), though absolute values were 12% lower on average (p<0.001), and inversely related to obesity measures, particularly BMI (r=-0.29, p=0.001) (Table 2.2). There were no significant associations between 0900h ACTH or cortisol concentrations and age, adult lifestyle factors or depression score.

ACTH and cortisol concentrations rose in all subjects following CRH administration. ACTH peaked between 15 and 30 minutes and the cortisol peak followed at 45 to 60 minutes. Median ACTH and mean cortisol responses (iAUC) to CRH were 652 (IQR 344-1002) pmol/l.min and 13823 (sd 9881) nmol/l.min respectively. Both ACTH and cortisol

responses were positively correlated with obesity (Table 2.2). There were no relationships with age or lifestyle variables, apart from activity which was inversely associated with the response (ACTH iAUC rho=-0.23, p=0.02; cortisol iAUC: rho=-0.17, p=0.08). Depression score was not correlated with the response to CRH.

2.3.2.4 *DEX/CRH test*

The mean DST result was 23.2 (gsd 1.7) nmol/l. All subjects responded to CRH, but the range of responses was considerable: ACTH increment: 0.07-42.8 pmol/l (see section 2.2.5) and cortisol increment: 1-520 nmol/l. The peak occurred on average at 60 minutes for ACTH and 75 minutes for cortisol. The median ACTH and cortisol responses (iAUC) during the DEX/CRH test were 283 pmol/l.min (IQR 131-537) and 4264 nmol/l.min (IQR 986-13,534) respectively. There were no relationships between age or current size and the results of the DEX/CRH test (Table 2.2). There was a tendency for the cortisol response during the DEX/CRH test to be lower in men who consumed more than 10 units of alcohol per week (p=0.07), but none of the results were predicted by the other lifestyle variables. Cortisol response tended to be greater in those subjects with higher depression scores (rho=0.15, p=0.1).

Table 2.2 Correlation between measures of HPAA activity and age and obesity.

	Baseline assessment 0900h cortisol	Salivary cortisol iAUC	0900h ACTH	0900h cortisol	CRH test ACTH iAUC	CRH test cortisol iAUC	DST cortisol	DEX/CRH ACTH iAUC	DEX/CRH cortisol iAUC
N	678	117	119	121	113	114	103	101	101
Age	**0.10** (0.01)	0.10 (0.3)	-0.08 (0.4)	-0.03 (0.8)	0.00 (1.0)	-0.05 (0.6)	0.01 (1.0)	-0.16 (0.1)	-0.13 (0.2)
BMI	**-0.11** (0.003)	-0.17 (0.06)	-0.06 (0.5)	**-0.29** (0.001)	**0.24** (0.01)	**0.36** (<0.001)	-0.06 (0.6)	0.03 (0.8)	-0.05 (0.6)
WHR	**-0.09** (0.02)	**-0.19** (0.04)	-0.07 (0.5)	**-0.19** (0.03)	**0.20** (0.03)	**0.31** (<0.001)	-0.01 (0.9)	-0.03 (0.8)	-0.05 (0.6)
Body fat	**-0.09** (0.03)	-0.14 (0.1)	-0.05 (0.6)	**-0.19** (0.04)	0.09 (0.3)	**0.31** (<0.001)	-0.03 (0.7)	-0.08 (0.4)	-0.08 (0.4)
SSTR	-0.05 (0.2)	-0.16 (0.09)	-0.11 (0.2)	-0.09 (0.4)	0.12 (0.2)	0.10 (0.3)	-0.17 (0.09)	-0.01 (0.9)	0.05 (0.6)

Each cell contains a Pearson correlation coefficient and p value. Values in bold denote significant results.
iAUC: incremental area under the response curve
DST: Overnight 1.5mg dexamethasone suppression test
DEX/CRH: dexamethasone-suppressed CRH test
BMI: body mass index, WHR: waist to hip ratio, SSTR: subscapular to triceps ratio

2.3.3   Fetal programming of HPAA activity

2.3.3.1   Unstimulated HPAA activity

There was no relationship between birthweight and fasting 0900h cortisol concentration in the 678 men at their baseline assessment (r=0.01, p=0.9) (Figure 2.4). In keeping with this finding, there were no differences in baseline ACTH or cortisol concentrations prior to the CRH test between the two birthweight groups that participated in the HPAA study (Table 2.3). Subgroup analysis revealed similar results in the 103 men who completed the DEX/CRH test (data not shown).

2.3.3.2   Salivary cortisol response to awakening

Eighty-three percent of individuals showed a cortisol response to waking in both birthweight groups and the time of the peak response did not differ between the groups (p=1.0). Likewise, neither the total cortisol production in the first hour after waking (AUC) nor the magnitude of the response as assessed by the iAUC differed significantly in LBW and HBW men (Table 2.4).

2.3.3.3   CRH test

The ACTH and cortisol responses to CRH administration are shown in Table 2.5 and Figure 2.5. None of the indices of ACTH response differed between the two birthweight groups. There was a trend towards reduced cortisol response to CRH in the LBW group which was confirmed on longitudinal analysis (p=0.1), but there was no birthweight:time interaction – ie the response profiles did not separate with time. The analysis was repeated for the 103 subjects that completed the DEX/CRH test and the results were similar.

Figure 2.4 Relationship between birthweight and fasting 0900h cortisol
concentration in 678 men aged 64 years.
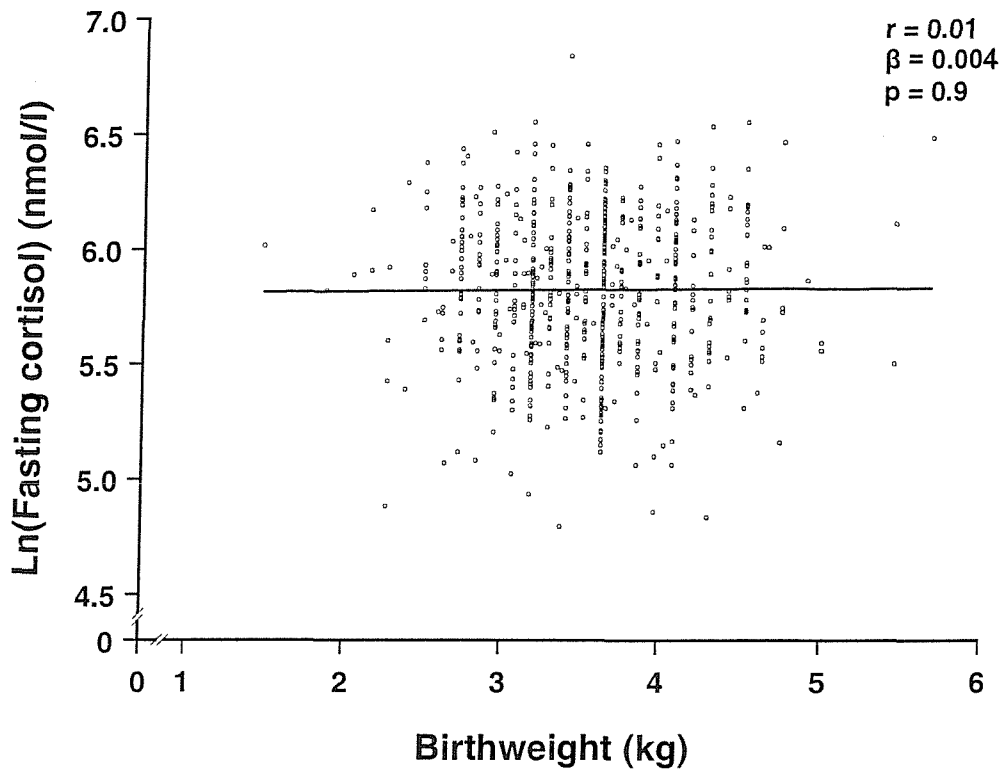
Table 2.3   Unstimulated HPAA activity in LBW and HBW men.

Birthweight group	0900h plasma ACTH (pmol/l)	0900h serum cortisol (nmol/l)
LBW	6.4 (1.6)	302 (1.4)
N	*57*	*58*
HBW	5.6 (1.6)	306 (1.3)
N	*62*	*63*
All	6.0 (1.6)	304 (1.3)
N	*119*	*121*
p	0.1	0.8
p^a	0.2	0.5

Data are geometric mean (sd) *n*
[a]corrected for BMI
LBW: low birthweight, HBW: high birthweight

Table 2.4   Cortisol response to awakening in LBW and HBW men.

Birthweight group	Salivary cortisol AUC (nmol/l.min)	Salivary cortisol iAUC (nmol/l.min)
LBW	1037 (393)	281 (428)
N	*56*	*56*
HBW	1082 (361)	283 (416)
N	*61*	*61*
All	1060 (375)	282 (420)
N	*117*	*117*
p	0.5	1.0
p[a]	0.5	0.8

Data are mean (sd) *n*
[a]corrected for BMI
AUC: area under the response curve, iAUC: incremental area under the response curve
LBW: low birthweight, HBW: high birthweight

Table 2.5   ACTH and cortisol responses to CRH stimulation in LBW and HBW men.

Birthweight group	ACTH iAUC[a] (pmol/l.min)	Cortisol iAUC[b] (nmol/l.min)
LBW	636 (323-939)	12557 (7942)
n	*52*	*53*
HBW	652 (392-1135)	14923 (11250)
n	*61*	*61*
All	652 (344-1002)	13823 (9881)
n	*113*	*114*
p	0.2	0.2
p[c]	0.2	0.3

[a] median (interquartile range) *n*
[b]mean (sd) *n*.
[c]corrected for BMI
iAUC: incremental area under the response curve
LBW: low birthweight, HBW: high birthweight

Figure 2.5 ACTH and cortisol profiles (mean, sem) during the CRH study.
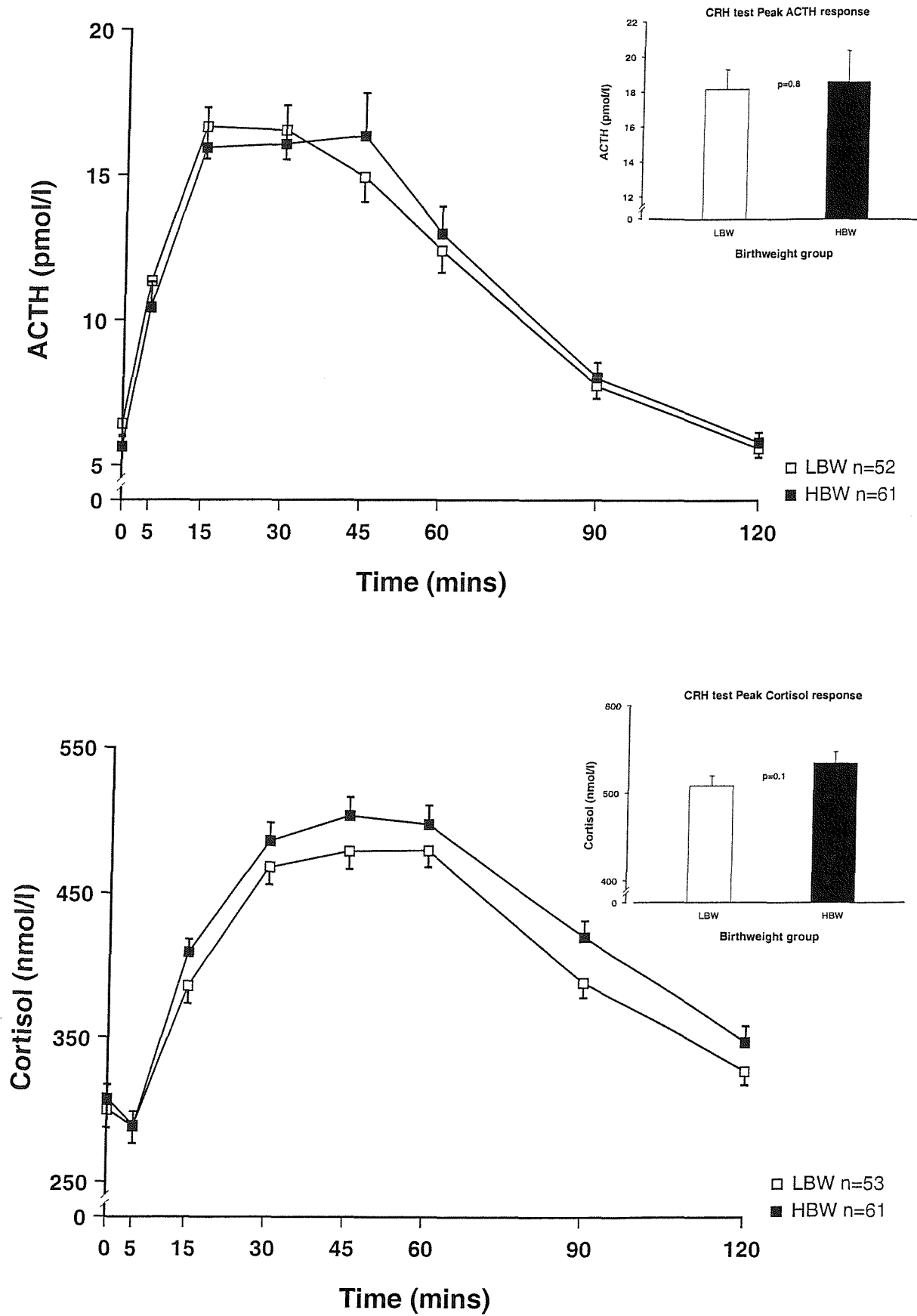
2.3.3.4   DEX/CRH test

Two subjects, one in each birthweight group, had a post-dexamethasone cortisol concentration of greater than 110nmol/l, the cutoff for non-suppression after a 1.5mg DST (152). There were no features to differentiate these men from the remainder, in particular their HAD scores were similar and their CRH test results were unremarkable, and they were therefore included in the analysis. Excluding them increased the significance of the findings described below.

Results of the DST and subsequent responses to CRH are shown in Table 2.6 and Figure 2.6. There were no significant differences in the baseline post-dexamethasone results between the two birthweight groups. However, both ACTH and cortisol responses to CRH stimulation after dexamethasone were lower in the LBW men. These differences were not altered by correction for potential confounders. Longitudinal analysis confirmed these findings and for cortisol there was a significant birthweight:time interaction (p<0.001).

Table 2.6   Post dexamethasone baseline and CRH-stimulated HPAA activity in LBW and HBW men.

	DST ACTH[ac] (pmol/l)	DST cortisol[a] (nmol/l)	ACTH iAUC (pmol/l.min)[bc]	Cortisol iAUC (nmol/l.min)[b]
LBW (n=46)	1.01 (2.0)	23.3 (1.7)	211 (83-514)	2,754 (795-8,963)
HBW (n=57)	1.14 (2.0)	23.2 (1.7)	341 (203-608)	6,840 (986-20,659)
All (n=103)	1.08 (2.0)	23.2 (1.7)	283 (131-537)	4,264 (986-13,534)
p	0.4	1.0	**0.02**	**0.05**

[a]geometric mean (SD)
[b]median (interquartile range)
[c]see section 2.2.5
DST: 1.5mg overnight dexamethasone suppression test
iAUC: incremental area under the CRH response curve
LBW: low birthweight, HBW: high birthweight

Figure 2.6 ACTH[a] and cortisol profiles (mean, sem) during the DEX/CRH study.
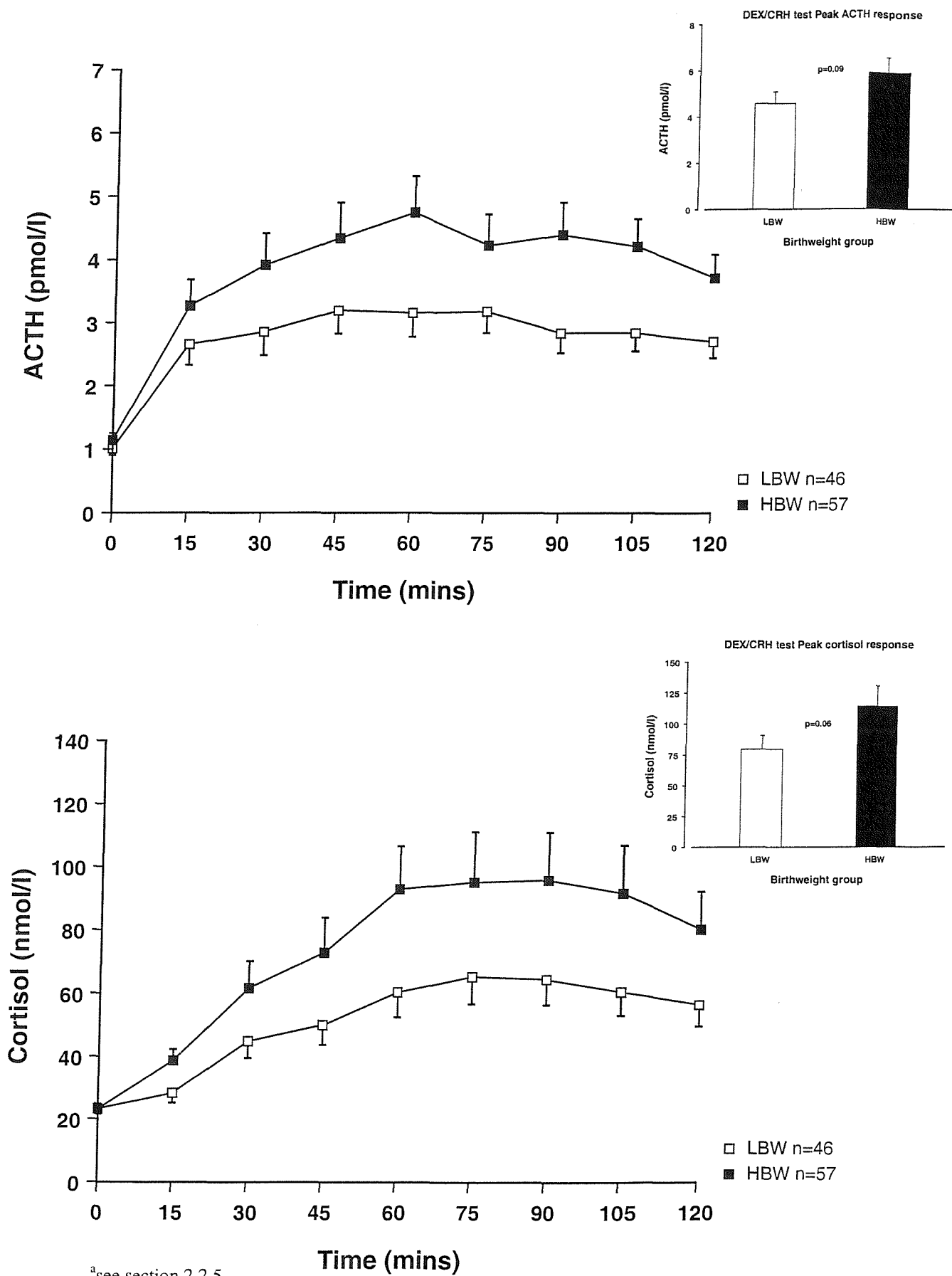
2.3.4 Relationship of HPAA activity to components of the metabolic syndrome

2.3.4.1 Baseline assessment

Table 2.7 details the mean values for the haemodynamic and metabolic variables

measured at the baseline assessment in the 678 men with a fasting 0900h cortisol

concentration. Partial correlation coefficients, corrected for age and BMI, were used to

assess the relationships between these variables and 0900h plasma cortisol concentration

in this group. Subjects with higher fasting cortisol had higher systolic (r=0.11, p=0.003)

and diastolic blood pressure (r=0.08, p=0.047), higher fasting (FPG) (r=0.15, p<0.001)

and post load glucose (120 minute sample after OGTT) (r=0.10, p=0.01), but there were

no differences in fasting insulin concentration, insulin resistance (HOMAr) or fasting

triglyceride and cholesterol concentrations (Table 2.8). There was an inverse relationship

between cortisol concentration and insulin secretion (r=-0.15, p<0.001). These

associations were not altered by correcting for adult lifestyle factors. Excluding the

subjects with diabetes (n=77) from these analyses removed the relationship with post load

glucose and weakened that with FPG (r=0.08, p=0.04), but the other associations were

unchanged.

Table 2.7   Haemodynamic and metabolic variables in the whole cohort and the HPAA study subgroup.

	Baseline assessment	HPAA study
N	678	122
Systolic BP (mmHg)	137 (19)	135 (17)
Diastolic BP (mmHg)	76 (11)	75 (10)
0900h cortisol (mmol/l)[a]	339 (1.4)	343 (1.3)
Fasting glucose (mmol/l)[a]	5.9 (1.2)	5.5 (1.1)[b]
120 min glucose (mmol/l)[a]	6.5 (1.5)	5.7 (1.4)[b]
Fasting insulin (pmol/l)[a]	69.0 (2.0)	58.6 (1.9)[b]
HOMAr[a]	3.6 (2.0)	2.9 (1.8)[b]
HOMAβ[a]	118 (1.8)	119 (1.7)
Total cholesterol (mmol/l)	6.0 (1.0)	5.9 (1.0)
Triglyceride (mmol/l)[a]	1.4 (1.6)	1.3 (1.6)
HDL cholesterol (mmol/l)[a]	1.3 (1.3)	1.3 (1.3)
Diabetes (%)	11.4	0[b]
Metabolic syndrome (%)	8.4	4.1

Data are mean (sd) [a]geometric mean (sd)
[b]Significant difference between those in the HPAA study and those not ($p < 0.01$)
BP: blood pressure
HOMAr: *homeostasis model assessment insulin resistance*
HOMAβ: homeostasis model assessment insulin secretion
HDL: high density lipoprotein

65

Table 2.8 Relationship between fasting 0900h cortisol and cardiovascular risk factors in 678 men aged 64yrs.

Cortisol quintiles (nmol/l)	N	Age (yrs)	BMI (kg/m^2)[a]	WHR	Systolic BP (mmHg)	Diastolic BP (mmHg)	Fasting Glucose (mmol/l)[a]	120 min Glucose (mmol/l)[a]	Insulin resistance (HOMAr)[a]	Insulin secretion (HOMAβ)[a]	Triglyceride (mmol/l)[a]
<259	136	63.9	27.4	0.97	134	76	5.8	6.4	4.0	140	1.5
-314	136	64.1	27.1	0.96	137	76	5.8	6.3	3.6	125	1.5
-371	136	64.4	26.3	0.95	137	76	5.9	6.2	3.5	113	1.3
-453	136	64.7	26.1	0.95	137	76	5.9	6.6	3.4	110	1.4
>453	134	64.5	26.5	0.96	140	78	6.1	6.9	3.6	106	1.4
All	678	64.3	26.7	0.96	137	76	5.9	6.5	3.6	118	1.4
SD		2.6	1.1	0.06	19	11	1.2	1.5	2.0	<0.001	0.6
p		0.01	0.003	0.02	0.005	0.1	0.001	0.04	0.2	<0.001	0.6
p[b]					0.003	0.047	<0.001	0.01	0.9	<0.001	0.5

Data presented are mean values for each cortisol quintile, [a]geometric mean; p values based on continuous analysis, [b]adjusted for age and BMI
BMI: body mass index, WHR: waist hip ratio, BP: blood pressure

The impact of obesity on the relationship between cortisol and cardiovascular risk factors was also examined. There was a significant interaction between cortisol and BMI in determining FPG concentrations (p=0.009) (Figure 2.7). The correlation between cortisol and FPG increased across BMI tertiles (BMI <25.2 r=0.09 (p=0.2), BMI 25.2-28 r=0.11 (p=0.1), BMI ≥28 r=0.20 (p=0.002)). Cortisol and obesity were independent predictors of the other cardiovascular risk factors, but the interaction terms were not statistically significant.

Figure 2.7 Interaction of 0900h cortisol concentration and BMI in predicting fasting plasma glucose.
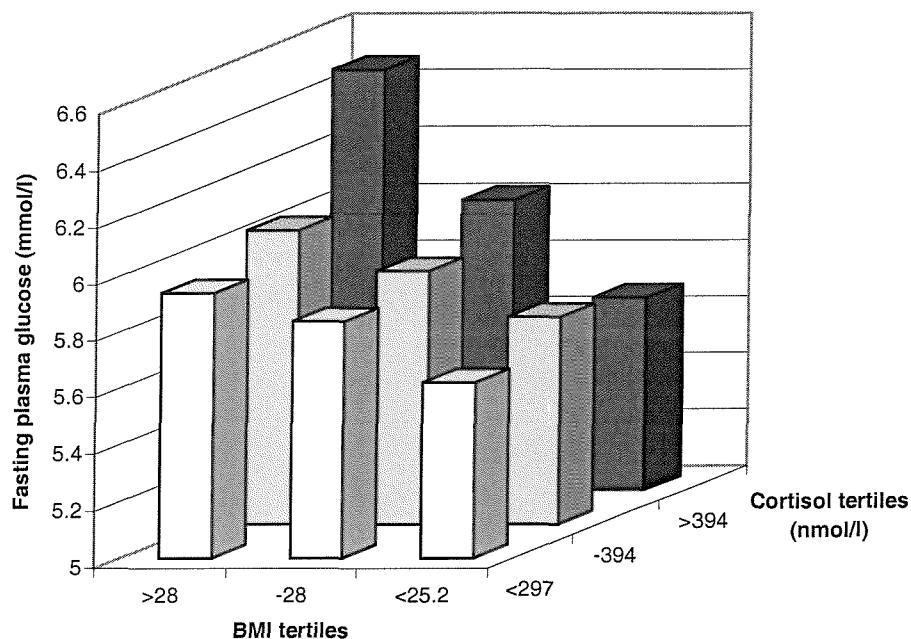
The prevalence of diabetes and the metabolic syndrome was increased in those subjects with the highest cortisol concentrations (Table 2.9). The odds of diabetes increased 1.6-fold (p<0.001) for each SD increase in fasting 0900h cortisol, while those of the metabolic syndrome increased 1.5-fold (p=0.004). The corresponding effects of an SD increase in BMI were 2-fold and 1.75-fold respectively (p<0.001).

Table 2.9   Prevalence and odds ratios of diabetes and the metabolic syndrome according to 0900h cortisol tertile.

Cortisol tertiles (nmol/l)	N	Diabetes n (%)	Odds ratio (95% CI)	Metabolic syndrome n (%)	Odds ratio (95% CI)
<297	226	18 (8.0)	1.0	16 (7.1)	1.0
-394	226	21 (9.3)	1.5 (0.8-3.0)	14 (6.2)	1.0 (0.5-2.2)
>394	226	38 (17.1)	3.1 (1.6-5.7)	27 (12.0)	2.0 (1.0-4.0)
Total	678	77 (11.4)		57 (8.4)	
p[a]			<0.001		0.004

[a]based on continuous analysis, corrected for age and BMI

2.3.4.2   HPAA study

The 122 subjects who took part in the HPAA study did not differ from the cohort as a whole in age, anthropometry, lifestyle variables (Table 2.1) or the majority of the cardiovascular risk factors measured at baseline (Table 2.7). Glucose tolerance and insulin sensitivity were better in this subgroup, as expected given that diabetes was an exclusion criterion for the HPAA study. However, the proportion of men with impaired glucose tolerance (2hr glucose 7.8-11.0 mmol/l) was similar amongst those who took part in the study and those who did not (16.5% vs 20.4% p=0.3). The participants were not a random sample of the cohort (the programming study required a factorial design as described above), but there were no significant differences between individuals in the top and bottom birthweight quartiles, who could have been selected for the HPAA study, and those in the middle quartiles. In addition, correcting for birthweight group did not influence any of the results described below.

BMI and WHR were closely correlated in the HPAA study group (r=0.74, p<0.001) and it was not possible to define a group of men who differed in WHR for a given BMI to assess the effect of central versus peripheral obesity. BMI was a stronger predictor of the cardiovascular risk factors than WHR; systolic blood pressure: r=0.36 vs r=0.34, diastolic blood pressure: r=0.32 vs r=0.27, fasting glucose: r=0.26 vs r=0.22, 2hr glucose: r=0.34 vs r=0.32 and triglycerides: r=0.37 vs r=0.34 and was used to assess the influence of obesity in all the analyses.

There were no associations between the parameters derived from the salivary cortisol profile over the first hour after waking and blood pressure or any of the metabolic variables (Table 2.10). Likewise, the cortisol response to CRH was not related to blood pressure, glucose tolerance, insulin sensitivity or plasma lipid concentrations in this cohort of 65yr old men (Table 2.10). Fasting plasma glucose and insulin resistance (HOMAr) were positively correlated with the ACTH response to CRH in univariate analysis, but these relationships did not withstand correction for age and obesity. Analysis of the results of the DEX/CRH test showed that neither the degree of dexamethasone suppression nor the subsequent ACTH and cortisol responses to CRH predicted the components of the metabolic syndrome (Table 2.10). Only five participants fulfilled the criteria for the metabolic syndrome and therefore logistic regression analysis of this categorical variable was not performed.

Table 2.10 **Associations between the HPAA study results and the components of the metabolic syndrome.**

	Salivary cortisol iAUC	0900h cortisol	CRH test ACTH iAUC	CRH test cortisol iAUC	DST cortisol	DEX/CRH ACTH iAUC	DEX/CRH cortisol iAUC
N	117	121	113	114	103	101	101
Systolic BP	0.01	-0.02	0.08	0.06	0.01	-0.10	-0.11
	0.06	0.11	-0.01	-0.07	0.02	-0.06	-0.06
Diastolic BP	-0.08	-0.02	0.12	0.11	0.05	0.02	-0.02
	-0.02	0.08	0.04	-0.01	0.07	0.02	0.00
Fasting glucose	0.00	0.02	**0.23**	0.11	-0.14	0.11	0.07
	0.03	0.10	0.18	0.04	-0.13	0.11	0.10
2hr glucose	0.01	-0.12	0.16	0.08	0.04	0.01	-0.05
	0.06	-0.02	0.09	-0.05	0.06	0.03	-0.01
Insulin resistance	-0.16	-0.12	**0.21**	0.17	0.02	0.09	0.03
	-0.08	0.05	0.09	-0.03	0.06	0.06	0.04
Insulin secretion	-0.17	-0.18	0.04	0.08	0.14	0.02	-0.03
	-0.09	-0.08	-0.06	-0.06	0.17	-0.02	-0.04
Triglyceride	-0.08	-0.06	0.09	0.10	-0.04	-0.07	-0.15
	-0.01	0.06	0.00	-0.05	-0.02	-0.09	-0.14

Each cell contains the Pearson correlation coefficient relating the two variables and beneath it the partial correlation coefficient corrected for age and BMI. Coefficients in bold denote a statistically significant association, $p < 0.05$
iAUC: incremental area under the response curve
DST: overnight 1.5mg dexamethasone suppression test
DEX/CRH: dexamethasone-suppressed CRH test
BP: blood pressure

2.4 DISCUSSION

2.4.1 Fetal programming of HPAA activity

This study has examined the effect of birthweight on central regulation of the pituitary-adrenal axis using three tests; awakening, a standard CRH test and a combined DEX/CRH test. It is important to recognise that all the measurements during these tests were peripheral and thus statements about central HPAA function have been inferred from the results, but cannot be definitive. The results show that LBW and HBW men have similar salivary cortisol responses to awakening, ACTH and cortisol responses to CRH and dexamethasone suppressibility, but that post-dexamethasone responses to CRH are reduced in this group of LBW men.

A factorial design was chosen, comparing individuals in the outer quartiles of birthweight, as performing the CRH tests on a large enough sample across the birthweight range would have been impractical. A clear weakness of this type of study is that it is not able to detect quadratic relationships. However, previous studies of HPAA activity in adults have shown linear relationships with size at birth. The groups chosen were not particularly extreme (50% of the population fell within the sampling frame), but there was sufficient separation between the mean birthweights in the two groups (2.8 vs 4.3 kg) that differences related to size at birth should have been apparent.

Salivary cortisol measurements provide a convenient means of assessing the HPAA axis outside the laboratory setting and avoid venepuncture-related stress responses. Using this method, reproducible rises in salivary cortisol concentration have been shown to occur thirty minutes after waking (162) and the same group have reported exaggerated responses in individuals under chronic stress (163). This measure appears to reflect a unique aspect

of HPAA function as it does not correlate closely with other tests of HPAA activity such as diurnal rhythm, CRH responsiveness and stress reactivity, but the mechanisms involved are not yet understood (165, 179). As the participants had all previously attended the same clinic for their baseline assessment, the waking response in the present study is unlikely to reflect anxiety in anticipation of the clinic attendance later that morning. A recent publication has raised the issue of compliance with home saliva sampling and suggested that electronic devices should be issued to participants to improve data quality (180). This was not done in the current study and the results should be interpreted accordingly.

Two recent studies have shown a strong influence of waking time on the subsequent rise in salivary cortisol, in contradiction of earlier work which reported that this was not a determinant of the response (181, 182). In the present study, there were no relationships between time of waking and the variables derived from the salivary cortisol profiles. This may be due to the narrow range of waking times; as the participants had to attend the clinic by 0800h, the majority woke between 0500h and 0700h – ie within the early waking groups of the studies cited above. No other anthropometric or lifestyle factors were found to be determinants of the salivary cortisol response to awakening. Age has previously been shown to be inversely related to the response (181), though this may also have been unapparent in the current study due to the narrow age range of the participants.

The only study to examine awakening cortisol response in relation to size at birth previously was performed in twins. It found a blunted cortisol rise in the first 30 minutes in individuals weighing less than 2500g at birth in comparison with those who were greater than 3500g (183). However, many of the low birthweight individuals in this study were preterm and they were also significantly lighter at birth than the LBW (<3100g)

group in the present study which found no differences in cortisol response to awakening or total integrated cortisol concentration over the hour between the two birthweight groups. This new data adds to published evidence from 24 hour profiles of cortisol secretion suggesting that there is no relationship between birthweight and non-stressed cortisol rhythm (115, 116).

There were also no significant group differences in the ACTH and cortisol responses to CRH indicating that in these LBW men the corticotroph is able to respond normally to CRH. Likewise, following dexamethasone, the baseline ACTH and cortisol concentrations were similar in the two groups, reproducing the results from the older Hertfordshire cohort where a 0.25mg dexamethasone suppression test was used (117). However, the LBW group had significantly lower ACTH and cortisol responses to CRH testing after dexamethasone. It is possible that this finding reflects differential dexamethasone metabolism in the two birthweight groups. However, this is thought to be unlikely as there was no difference in dexamethasone concentration after an overnight DST in the highest and lowest birthweight quartiles in the previous study and correcting for dexamethasone concentration did not alter the birthweight HPA associations in that study(117). In the present study, no patient was taking medication known to influence the activity of hepatic CYP3A4 activity and thus dexamethasone metabolism.

A fixed dose of CRH (100µg) was used throughout this study. This is in line with standard European practice for both clinical and experimental use of the CRH test and is the only dose that has been published for the DEX/CRH test. As mean current weight differed between the two birthweight groups, the LBW men were exposed to a slightly higher dose of CRH per kilogram on average (LBW: 1.3µg/kg, HBW: 1.2µg/kg). The dose response

curve for CRH suggests that 1µg/kg produces a maximal cortisol response (147), and therefore the small difference in average dose is unlikely to have influenced the results of the study. The fact that the LBW group had lower ACTH and cortisol responses in the DEX/CRH test despite receiving a slightly higher dose of CRH increases the significance of the result.

The DEX/CRH test has been developed as a tool to investigate subtle HPAA dysfunction particularly in depression and related pathological conditions. In line with several studies showing that depression is associated with increased CRH responses following dexamethasone (149, 152), the current data revealed a trend towards greater responses in subjects with higher HAD scores, despite none reaching the threshold for clinical depression. As dexamethasone does not freely pass the blood brain barrier its major target is thought to be the pituitary gland (153). As outlined in the introduction to this chapter, it is suggested that the DEX/CRH test findings in depression reflect decreased pituitary sensitivity to dexamethasone, possibly as a result of increased vasopressin action at the corticotroph which attenuates the inhibitory effect of glucocorticoids on CRH-stimulated ACTH secretion. The lower response in LBW men may therefore reflect the reverse; increased pituitary sensitivity to dexamethasone or reduced vasopressinergic drive.

Previous studies have suggested that people who were small at birth have high urinary excretion of glucocorticoids in childhood, raised fasting plasma cortisol concentrations in adult life and increased adrenal responsiveness to synthetic ACTH administration (111, 112, 117, 119). As similar abnormalities in melancholic depression are thought to be due to abnormal central regulation of the pituitary-adrenal axis, it was hypothesised that responses to CRH testing would be similar to those seen in depressed patients. However,

the pattern observed bears closer resemblance to the alterations in HPAA activity associated with chronic fatigue and atypical depressive syndromes, such as seasonal affective disorder. Although there are no studies using the DEX/CRH test, Demitrack and colleagues found reduced ACTH and cortisol responses to CRH in patients with chronic fatigue syndrome, which they suggested reflected impaired central drive to the pituitary (184). Other groups have confirmed these findings and similar results have been obtained in studies of subjects with seasonal affective disorder (185, 186).

The present study was designed to continue work begun in the older Hertfordshire cohort in which a strong relationship between fasting 0900h cortisol concentration and birthweight had been found. However, as these individuals are now in their 70's and 80's, it was felt inappropriate to involve them in this series of investigations and therefore members of the 1931-1939 cohort were recruited. The baseline data for the new cohort was not available until after the HPAA study was complete. As outlined in the results, there was no relationship between 0900h cortisol concentration and birthweight in the 678 men who form the new East Hertfordshire cohort and thus the results of the present study cannot be used to explain the nature of the HPAA abnormality in hypercortisolaemic low birthweight individuals.

A detailed comparison of the two cohorts has yet to be undertaken and the candidate was not at liberty to analyse data from the new cohort, other than that directly relating to the HPAA study. The original report of an inverse association between birthweight and 0900h cortisol concentration was based on 370 men born in the same area of Hertfordshire between 1920 and 1930 (110). These men were the same age as those in the new cohort when they were studied (mean 64 years, range 59 - 70 years). Likewise their birthweights

were virtually identical (Old: mean 3.5kg (range 1.4 - 5.2), New: mean 3.5kg (range 1.5 - 5.7)), as were their 0900h cortisol concentrations (Old: mean 344nmol/l (range 112 - 702), New: mean 338nmol/l (range 121 - 702)). Mean BMI was also very similar although the ranges did differ slightly (Old: mean 26.9 (range 16.8 - 41.7), New: mean 26.7 (range 18.2 - 46.9)).

The apparent similarity between these two groups of men makes the difference in the relationship between birthweight and 0900h cortisol all the more striking. One possible explanation is that there were different environmental influences on birthweight in the two decades. The paucity of early life data in these cohorts renders detailed investigation of this hypothesis impossible. Analysis of the relationships between birthweight and cardiovascular and metabolic variables is ongoing, but should provide an insight into whether there are other generational differences in programming.

Other recent data suggest that there may be heterogeneity in the HPAA abnormalities associated with low birthweight. A study in Finland found that in subjects born before 39 weeks of gestation there was an inverse correlation between birthweight and cortisol, whereas in subjects born after 40 weeks the correlation was positive (113). Unfortunately, reliable gestational age data is unavailable in the Hertfordshire cohorts preventing similar analysis from being undertaken. Animal studies have repeatedly shown that different long term effects on HPAA function are achieved by altering the nature and timing of the prenatal insult and it is likely that programming effects are similarly diverse in humans (76, 187).

In addition, birthweight is a crude indicator of antenatal exposures and, in both animal and human studies, prenatal insults have been shown to affect HPAA activity independently of birthweight. For example, in a group of young Scottish adults whose mothers were advised to eat a high protein, low carbohydrate diet during pregnancy, there was no association between birthweight and fasting morning cortisol, but higher meat/fish intake in late pregnancy was associated with raised cortisol concentrations at 30 years of age (109). Future studies in this field will be aided by new prospective cohorts where detailed data has been collected before, during and after pregnancy.

In conclusion, this study adds to the evidence that low birthweight is associated with subtle abnormalities of HPAA function but does not explain the alterations in HPAA activity previously reported to be associated with low birthweight.

2.4.2 HPAA activity and the metabolic syndrome

Several large cross sectional studies have examined the relationship between fasting plasma cortisol and cardiovascular risk factors (see sections 1.3.5 and 1.4.2.3) (62-64, 110, 111). In men, the strongest relationships tend to be between cortisol and blood pressure, whereas in women insulin sensitivity and lipid concentrations are closer correlates of cortisol. The effect of obesity differs in these studies, in general adding to the effect of raised cortisol concentrations, but evidence of an interaction between cortisol and obesity has been found in some cohorts (111). Data from the baseline assessment of the new Hertfordshire cohort, kindly made available for this thesis, therefore support previous work in this field. Fasting 0900h cortisol concentrations were inversely related to obesity measures and positively correlated with blood pressure and glucose tolerance. Cortisol and

BMI interacted in predicting fasting plasma glucose and the odds of having diabetes or the metabolic syndrome were increased in those individuals with the highest fasting cortisol.

The data from the detailed HPAA study was used to explore the possible mechanisms underlying these associations in a large number of men. Previous studies have focussed mainly on the effects of CRH stimulation in different patterns of obesity using small case control studies. Initial reports suggested that the ACTH response to CRH was blunted in obesity, but these results were not reproduced in a subsequent larger study from the same group (169, 188). Women with abdominal obesity have elevated ACTH and cortisol responses to CRH (189). In men, however, published data vary with one group reporting exaggerated ACTH responses in generalised obesity, while others have found a reduced cortisol response in men with a high WHR (190, 191). The current study has revealed strong positive correlations between three measures of obesity (BMI, WHR and percentage body fat) and cortisol responses to CRH. Slightly weaker relationships with the ACTH response were present for BMI and WHR. A study of thirteen men in this age group found that SSTR was the only obesity measure to predict CRH responses (192). There were no associations between SSTR and any parameter derived from the CRH test or any of the metabolic variables in the current study. The close correlation between BMI and WHR in this group of 65-year old men prevented any subgroup analysis of the differences between central and peripheral obesity.

Little data has been published relating the response to CRH stimulation to the components of the metabolic syndrome. One study found an association between ACTH response and insulin resistance (assessed by HOMA), but another found no relationship between either ACTH or cortisol responses and M/I insulin sensitivity index during a hyperinsulinaemic

euglycaemic clamp (190, 193). Bano and colleagues compared two groups of Asian women matched for BMI and found no difference in CRH response in those with and without diet-controlled type 2 diabetes (194). The present data support these findings, showing that cortisol responses to CRH did not relate to blood pressure, glucose tolerance, insulin sensitivity or lipid concentrations in a large group of men. There was a statistically significant positive association between the ACTH response to CRH and both fasting plasma glucose and insulin resistance in univariate analysis. However, these relationships were weakened considerably by correction for obesity and given the large number of analyses performed these significant findings may represent Type 1 errors.

Overall, the results of the current study imply that alterations in corticotroph number and sensitivity to CRH are not responsible for the association between raised circulating cortisol concentrations and the components of the metabolic syndrome. In addition, extrapolating from the results of CRH tests in patients with major depression, it appears that CRH drive to the pituitary is not increased in men with cardiovascular risk factors.

The salivary cortisol response to awakening and the DEX/CRH test have not previously been used in this area of research. Whilst the response to awakening (iAUC) was inversely associated with obesity measures, it did not predict any of the metabolic variables and nor were there any associations between the total integrated salivary cortisol concentration over the first hour and cardiovascular risk factors. Cortisol response in the DEX/CRH test has been shown to increase with age, but there are no references to associations with obesity in the literature. This study revealed no relationship between anthropometric measures and ACTH or cortisol responses to CRH after dexamethasone suppression.

Likewise, there were no associations with any of the haemodynamic or metabolic variables.

There was no association between the dexamethasone suppression test result in the current study and BMI or WHR. The literature is divided on the effects of dexamethasone suppression in obesity, with some studies showing enhanced and others showing reduced suppression (195). On balance, full dose dexamethasone (1mg or above) as used here does not distinguish between obese and non obese individuals, but there may be some subtle disturbances revealed when using low dose DSTs. Ljung and colleagues showed an inverse relationship between WHR and the degree of suppression using 0.5mg dexamethasone, but there were no relationships with BMI in their study (196). Conversely, in the older Hertfordshire cohort higher WHR was associated with lower plasma cortisol concentrations after 0.25mg dexamethasone (117). In the same study, Reynolds and colleagues found no association between low dose dexamethasone suppressibility and the components of the metabolic syndrome, results repeated in the present study.

It is noteworthy that participants with diabetes were excluded from the HPAA study and thus the range of glucose tolerance was necessarily restricted. This may have reduced the likelihood of finding associations between pituitary-adrenal responses to the challenge tests and cardiovascular risk factors. In the older Hertfordshire cohort, where strong associations between adrenal responsiveness to low dose ACTH and disease were found, individuals with diabetes were included (117). However, in the current study, the relationships between fasting 0900h cortisol concentration at the baseline assessment and the components of the metabolic syndrome remained even after exclusion of those

individuals with diabetes, suggesting that this was a suitable group in which to perform a study looking for possible mechanisms behind these associations.

In conclusion, this study suggests that associations between higher morning cortisol concentrations and the components of the metabolic syndrome are not due to enhanced pituitary CRH responsiveness. In addition, while accepting that no definitive statements about central control of the HPAA can be made when only peripheral measurements have been taken, exaggerated CRH and AVP drive to the pituitary do not appear to be responsible for these associations. The rationale behind this statement, which is derived from the interpretation of CRH and DEX/CRH test in patients with major depression, has been described in the introduction of this chapter and in the programming section of the discussion and thus is not reiterated.

Other mechanisms that may link glucocorticoids and the metabolic syndrome include altered cortisol clearance or increased tissue sensitivity (193, 197). Alternatively, as a serum cortisol concentration measured on the first visit to an unfamiliar clinic may represent a stress response, a suggestion supported by the significantly lower cortisol concentrations at the second visit in the HPAA study participants, it is possible that individuals with heightened stress reactivity may go on to develop cardiovascular risk factors. The data presented in this chapter suggest that future research should be concentrated in these areas.

2.5 SUMMARY

1) Comparing a group of LBW and HBW men there were no differences in

   - The salivary cortisol response to awakening

   - 0900h ACTH and cortisol concentration

   - ACTH and cortisol responses to 100μg human CRH

   - ACTH and cortisol concentrations after an overnight 1.5mg DST

2) LBW men had reduced ACTH and cortisol responses to CRH following dexamethasone suppression.

3) There were no associations between the components of the metabolic syndrome and

   - The salivary cortisol response to awakening

   - ACTH and cortisol responses to 100μg human CRH

   - ACTH and cortisol concentrations after an overnight 1.5mg DST

   - ACTH and cortisol responses during a DEX/CRH test

CHAPTER 3

Fetal Programming of Stress Responses

3.1 BACKGROUND

Psychological stress is a potent central stimulus to the HPAA, acting principally via the

limbic system to promote hypothalamic CRH synthesis and release (53). As suggested in

Chapter 2, the association between size at birth and fasting 0900h plasma cortisol may

reflect heightened stress reactivity in low birthweight individuals, as similar relationships

have not been found with other measures of 'basal' HPAA activity (115). In animal

models, prenatal stress results in abnormal HPAA activity during challenging situations

later in life (76). To date, no human studies have directly assessed whether HPAA

responses to stress may be determined antenatally, but Nilsson and colleagues looked

retrospectively at a cohort of Swedish military recruits and found a positive correlation

between psychological functioning (assessed by questionnaire) and birthweight,

concluding that impaired fetal growth may be associated with increased stress

susceptibility (198).

In humans, the magnitude of the cardiovascular response to a psychological stressor varies

considerably between individuals, but these differences typically tend to be stable over

time (199). This observation has led to the formulation of the 'reactivity' hypothesis

which suggests that exaggerated cardiovascular responses to stress ultimately lead to

sustained hypertension through vascular remodelling and altered autoregulation (200).

This hypothesis is supported by prospective studies showing links between stress

responsiveness to standardised challenges and the prevalence of hypertension and related

pathologies, including carotid atherosclerosis and increased left ventricular mass (201).

The extent to which these differences in cardiovascular reactivity could be a result of prenatal factors is not known, but the idea that they may be is supported by evidence that growth retardation during fetal life may be associated with increased autonomic activation throughout the lifecourse; adults and children who were small at birth have higher pulse rates in some studies (130, 131).

There is some animal data to support the hypothesis that programming of haemodynamic stress responses may be important in the development of hypertension. A study of rats born to mothers fed a 6% casein (low protein) diet showed that the major effect of prenatal undernutrition was to elevate blood pressure responses to an olfactory stressor; resting blood pressure was only slightly elevated (202). In this experiment, blood pressure was measured using an implanted telemetry probe with rats free to mobilise around their cages. These findings raised the possibility that the blood pressure elevations previously reported in studies of maternal undernutrition in rats may have been stress responses rather than resting values, as animals have to be restrained during measurements by indirect tail cuff plethysmography (34). Animal studies have also suggested that sympathoadrenal responses may be programmed by an adverse early environment. For example, the offspring of rats exposed to noise and light stress during pregnancy showed heightened sympathetic activation in response to footshock (124).

This chapter documents the results of a study designed to examine the hypothesis that individuals who were small at birth may have increased adrenocortical and cardiovascular responses to psychological stress in early adulthood.

The protocol combined three frequently used stress tests; the Stroop colour-word interference task, mirror-drawing and public speaking. Haemodynamic and HPAA reactivity have generally been studied separately, dependent on the interests of the group involved. There is a wealth of evidence supporting the use of these three tests in the assessment of cardiovascular reactivity and aggregating responses over a number of tasks has been shown to improve reliability (199). Stimulating the HPAA is more difficult and results vary between studies (203). The best results are seen with the Trier Social Stress Test (TSST) which requires the subject to perform a public speech and mental arithmetic in front of an audience (204). However, this test has not been validated in haemodynamic studies. In addition, the Adelaide group responsible for maintaining the cohort were concerned that some participants might find it threatening, potentially compromising recruitment for future studies.

The protocol for the current study was adopted on the advice of Professor Andrew Steptoe (University College London, UK) who has extensive experience in the field of psychobiological research (205-207) and additional measures, such as the use of a two-way mirror, were included in an attempt to increase the stimulus to the HPAA. Assessment of the neuroendocrine response was based on salivary cortisol alone, as the Adelaide team were insistent that the study was non-invasive.

3.2 METHODS

3.2.1 Participants

3.2.1.1 The Adelaide Family Heart Study cohort

A cohort of 856 individuals, based on sequential births between June 1975 and July 1976 at the Queen Victoria Hospital in Adelaide, South Australia, was established in 1984 as part of a WHO collaborative study looking at ischaemic heart disease risk factors in children (208). Contact was made with the families of 1138 of the 2000 children targeted and thus the cohort comprises 75% of those traced and invited. 25% of those who declined lived some distance from Adelaide at the time.

In 1995, the potential for studying the effects of size at birth longitudinally in this cohort was realised and a large follow up study was undertaken. Birthweight had been recorded for 822 (432 male, 390 female) of the 826 singletons. Other information abstracted from birth records includes length, head circumference, placental weight and gestational age (calculated from the date of the last menstrual period) (209). The cohort has been maintained since then and subgroups have taken part in studies examining the relationship between size at birth and blood pressure, insulin sensitivity and HPAA activity (24, 111, 210).

3.2.1.2 Stress study

For the present study, the aim was to recruit a random sample of 100 men and 100 women from across the birthweight range. A formal power calculation was not performed due to a lack of suitable normative data, but with 200 subjects a correlation of r=0.2 can be detected with 80% power at the 5% significance level. The 822 singletons with known birthweight were assigned a random number and ordered according to this number in sex-

specific birthweight quintiles (men: <3026g, 3027-3352g, 3353-3638g, 3639-3900g and >3901g and women: <2940g, 2941-3230g, 3231-3440g, 3441-3708g and >3709g). It was anticipated that a significant proportion of the cohort would be unavailable for the study as they now live a considerable distance from Adelaide, but still use their parents' address for contact purposes. An initial approach was made to 100 men (the first 20 in each quintile) to assess the number of subjects that would need to be considered to achieve the desired study size. On the basis of this preliminary sample, the first 60 men and women in each quintile formed the study sample (n=600).

Thirty-five of these individuals (5.8%) had previously stated that they did not wish to partake in further studies and they were therefore not invited to participate. Initial contact was by letter and this was followed up with a further letter and a telephone call if the telephone number was available. Where current address was unknown or incorrect, efforts were made to trace individuals through the electoral roll, the electronic telephone directory or via their parents, whose addresses were available from previous studies. Forty-seven individuals (7.8%) were untraceable. Participants were ineligible for the study if they had any history of pituitary problems, adrenal disease or diabetes, a recent history of depression or glucocorticoid use (last 3 months for both) or were pregnant or breast feeding. Thirty-nine individuals (6.5%) were ineligible; the majority women who were pregnant or lactating. Twenty-five percent of the sample were unavailable as they were resident in remote South Australia, interstate or overseas. Of the remaining 328 individuals, 184 (56.1%; 104 male, 80 female) were recruited for the study. A full breakdown of the sample is given in Figure 3.1. The study was approved by the ethics committee of the Women's and Children's Hospital, Adelaide and the participants gave written informed consent.

Figure 3.1 Recruitment of participants for the stress study.

Initial sample
N=600
(M:300, F:300)

Unavailable
N=272 (45.3%)
(M:111, F:161)

Reason	M	F
Withdrawn	10	25
Untraceable	24	23
Ineligible[a]	7	32
Distance[b]	70	81

Available for study
N=328 (54.7%)
(M:189, F:139)

Not interested
N=144 (43.9%)
(M:85, F:59)

Reason	M	F
Declined	73	47
Implied no[c]	12	12

Studied
N=184 (56.1%)
(M:104, F:80)
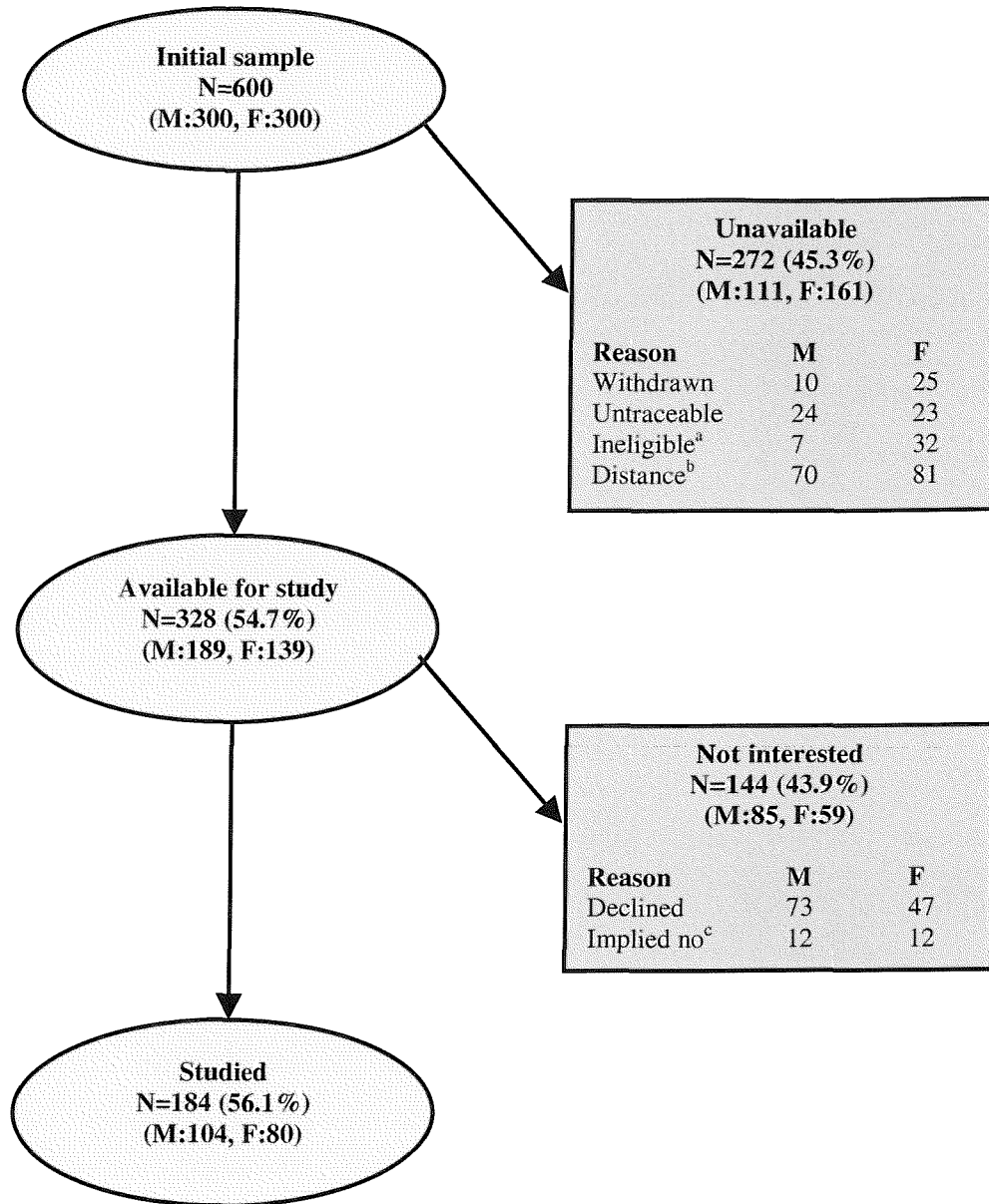
[a]Pituitary or adrenal disease, diabetes, recent depression or glucocorticoid use, pregnancy or lactation
[b]Remote South Australia, interstate or overseas
[c]No response to 2 letters to the correct address and no telephone number

3.2.2 Stress Protocol

Three investigators undertook the stress testing, though the majority of studies (62% male, 85% female) were performed by a single investigator. The study took place in the afternoon. The participants were asked to have lunch before the study and to abstain from alcohol, cigarettes, caffeine and vigorous exercise for two hours before arriving. They were also asked to have no more than two alcoholic drinks during the evening before the study. On arrival, their weight was measured to the nearest 0.1kg using digital scales (A & D Co. Ltd, Australia) and height to the nearest 0.1cm using a Holtain stadiometer (Holtain Ltd, Dyfed, UK). Waist and hip circumferences were also measured to the nearest 0.1cm.

Subjects were then taken to the room in which the stress testing was performed. They were seated in a comfortable armchair behind a desk and one investigator remained in the room throughout the study. Beat to beat monitoring of finger blood pressure and pulse was performed using a Portapres model-2 (TNO Biomedical Instrumentation, Amsterdam). A cuff, appropriate to the size of the finger, was placed on the third or fourth finger of the non-dominant hand (Plate 1). After fitting the cuff, a trial reading was performed to ensure that it was correctly positioned. The subjects were then informed that the study was about to commence and that talking with the investigator was not permitted, unless directly related to the protocol, due to the effect of speech on blood pressure. A continuous recording of pulse and blood pressure was made from the start of the study and the event marker was used to pick out important points in the protocol. Seven saliva samples were collected over the course of the study using the Salivette sampling device (Sarstedt, Leicester, UK). Swabs were placed in the mouth for two minutes and subjects were asked to try and ensure that they were saturated with saliva.

Figure 3.2 Schematic representation of the stress study protocol.

REST (20mins)		STRESS (40 mins)						RECOVERY (30 mins)		
Questionnaire		Stroop		Mirror		Speech			Questionnaire	
S	S		S		S			S	S	S
	M	M		M			M			M
	D						D			D

S Saliva sample
M Mood/task impact questionnaire
D Dinamap blood pressure recording
☐ Subject sitting quietly
▨ Preparation for task
▧ Stressor

Plate 1   Portapres finger cuff.
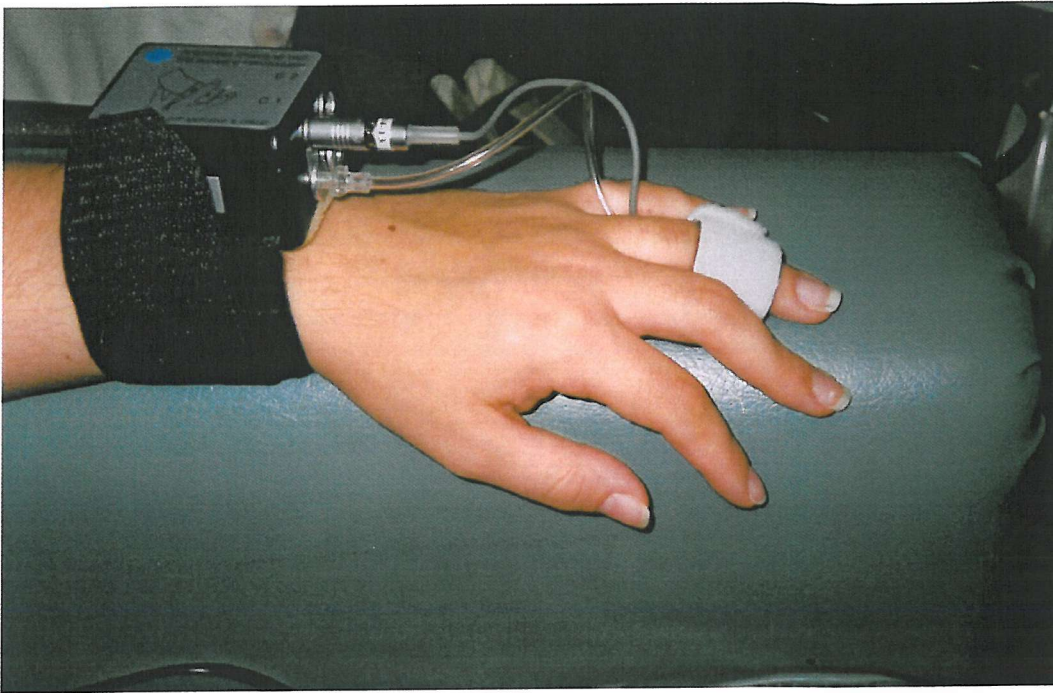
Plate 2   Stroop test.
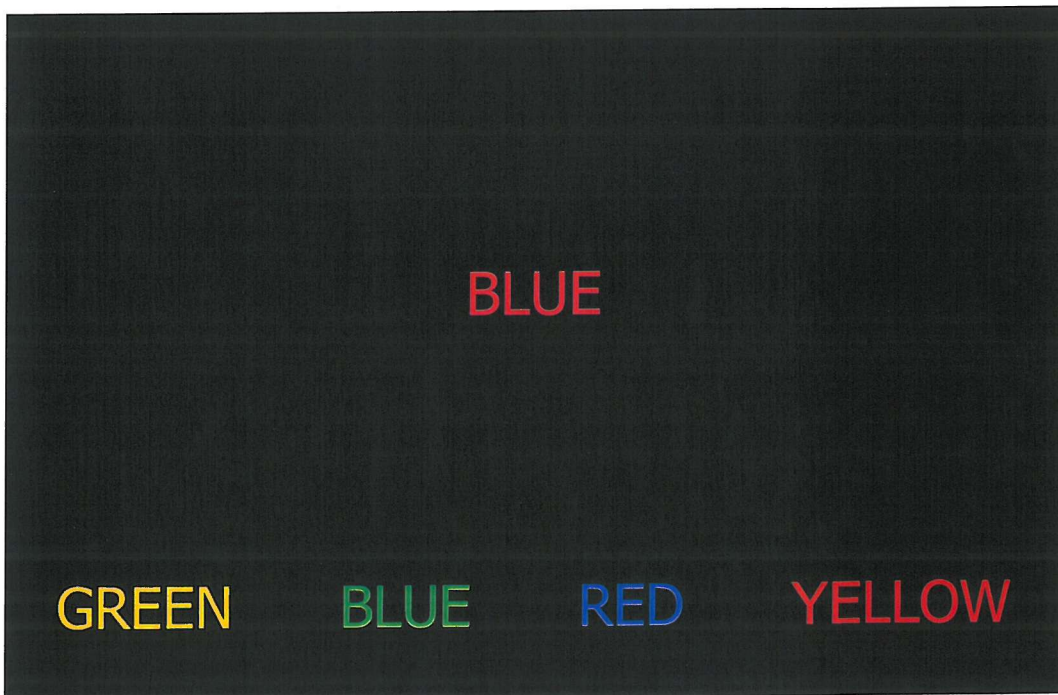
Plate 3   Mirror drawing.
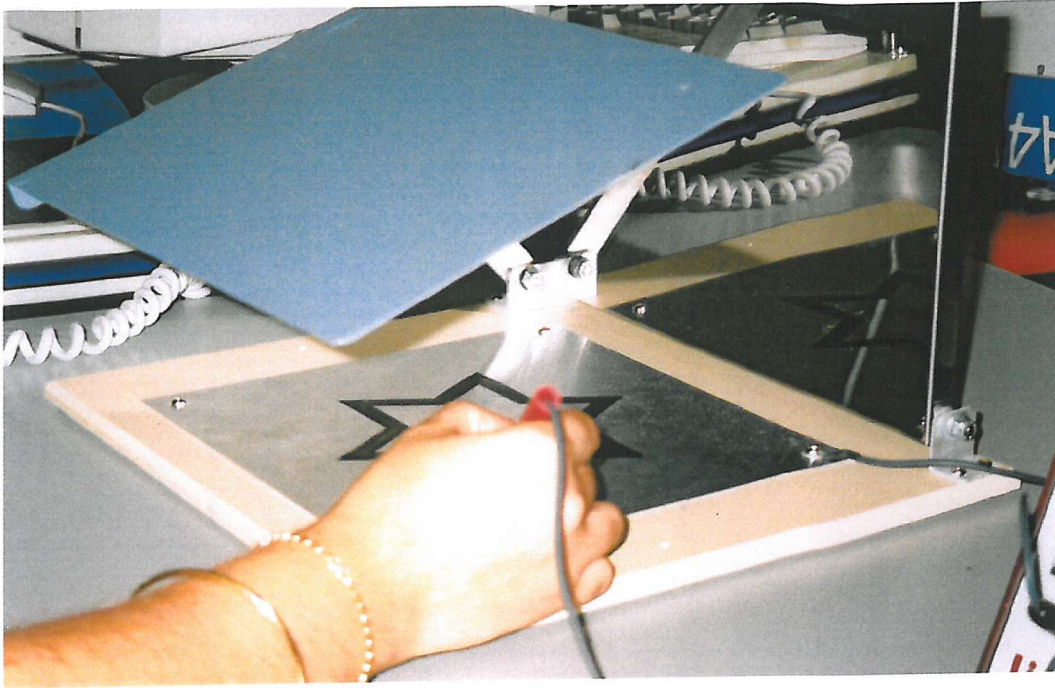
Plate 4   Speech task.

The protocol comprised three phases: rest, stress testing and recovery (Figure 3.2). During the 20 minute rest period, subjects completed a questionnaire on their health, education, employment, smoking and alcohol consumption, exercise patterns and mood (Centre for Epidemiologic Studies Depression Scale (CES-D) (211)) (Appendix B). An index of relative socioeconomic disadvantage (IRSD) was derived from their postcode (212). The mean score for the population is set at 1000 and a lower score denotes greater deprivation. For the final five minutes of the rest period, the subject was asked to sit quietly and the questionnaire was removed if not already completed (the majority had completed the questionnaire before this point). Saliva samples were taken at 5 and 20 minutes. At the end of the rest period, subjects were asked to rate how relaxed, anxious and stressed they felt on a scale of 1 to 7 (Appendix C).

The subjects then undertook three standard psychological stress tests, each lasting five minutes. They were given written and oral instructions and allowed a brief practice before commencing each task. Immediately after the tasks, subjects were asked to complete a task impact questionnaire, grading the difficulty of the task, their involvement in it, their performance, the degree of control they had over the task and how stressed and relaxed had felt on a scale of 1 to 7 (Appendix C). During the six minute recovery period between tasks, subjects were asked to sit quietly and a saliva sample was collected in the final two minutes of this period.

The first stressor was a computer-based colour-word conflict task; the Stroop test. In this task, the words red, blue, green or yellow appeared in the centre of the screen, printed in incongruent colours (Plate 2). Subjects were asked to ignore the word and to select the colour of the print from the four choices at the bottom of the screen. The order in which

these choices were presented and the colour in which they were written varied continuously. There was a finite time for making the selection and the computer provided the answer if the response was too slow or incorrect. The speed with which the words were presented varied throughout the test period, adding unpredictability to the task. At the end of the session, the number of words attempted and the number of correct responses were noted and the percentage of correct responses was calculated as a measure of performance.

The second task was a mirror-drawing test in which subjects were asked to trace the outline of a star that could only be seen in mirror image (Plate 3). The star was made of non-conducting material mounted on a metal plate and the stylus was metal-tipped (Lafayette Instruments Corp, Indiana, USA). Every time the stylus left the star a beep was heard and an error was recorded in the control unit. Subjects were asked to complete as many circuits as possible in five minutes, concentrating on accuracy rather than speed. They were advised that it is usual to complete five circuits in five minutes, influencing the degree of control that they felt over work pace. The number of circuits and errors were noted at the end.

The final stressor was a speech task. Subjects were faced with the hypothetical confrontational scenario of being unjustly accused of shoplifting by a store detective and having to defend themselves to the police. They were given two minutes to prepare a three minute speech in their defence. A second investigator entered the room at this point and a two-way mirror was exposed. The speech was videotaped and they were told that they could be observed from behind the mirror (Plate 4). They were also informed that their performance would be evaluated for fluency and confidence by communication experts.

During the final recovery period three saliva samples were collected: 10, 20 and 30 minutes after completion of the speech and the blood pressure and heart rate recording was continued throughout. After the speech, subjects were advised that the stress testing was complete and asked to sit quietly and relax for the first ten minutes. They then completed the questionnaire if necessary. For the final five minutes of the recovery period subjects were once again asked to sit quietly and then to rate how relaxed, anxious and stressed they felt on a scale of 1 to 7.

In addition to finger blood pressure, brachial pressure was measured using an automated oscillometric device (Dinamap PRO 100, Critikon Healthcare) at the end of the rest period, immediately after the speech and at the end of the recovery period. The average of three consecutive readings was taken each time. Prior to leaving the clinic subjects were debriefed, explaining in particular that there would be no further analysis of their speech performance as no recording had been made.

3.2.3   Diurnal variation in salivary cortisol concentration

In order to determine baseline adrenocortical activity in this cohort, the participants were asked to collect five further saliva samples, over the course of a normal working day, in the week following the clinic. Sample times were 30 minutes after waking, 0800h, 1200h, 1600h and 2200h. These samples were then posted back to the hospital. Compliance with this part of the study was poor; despite repeated follow up phone calls only 130 subjects (71%) sent back samples and many of these were collected some weeks after the clinic visit and at times other than those specified. A decision was made to analyse only the 1200h and 1600h samples to provide an estimate of adrenal activity at the time of day that the stress study was performed.

3.2.4 Laboratory methods

Salivettes were spun at 2500rpm for 10 minutes to recover the saliva from the swab and

the samples were then stored at -80^0C prior to assay for cortisol. They were analysed in

Adelaide by Dr David Kennaway, Associate Professor, Circadian Group, Department of

Obstetrics and Gynaecology, Adelaide University using a commercial kit (Salimetrics

LLC, State College, PA; interassay CV 6.69 – 6.88%).

3.2.5 Statistical analysis

Data from the Portapres was interpreted using software provided with the apparatus

(Beatscope 1.0, TNO Biomedical Instrumentation, Amsterdam). This generates a

continuous trace for finger blood pressure (systolic and diastolic) and heart rate and can be

used to calculate descriptive statistics over user-defined time periods. Blood pressure and

heart rate data were averaged between event markers during the rest, stress and recovery

periods. Further analysis was performed using Stata Statistical Software Release 7.0

(College Station TX: Stata Corporation).

Baseline blood pressure and heart rate were taken from the final five minutes of the rest

period. Mean blood pressure and heart rate during the three tasks was calculated (stressed

values), as aggregating responses over a number of tasks has been shown to produce a

more reliable measure of an individual's stress reactivity (199). The response to stress was

defined as the increment from baseline to stressed values.

The salivary cortisol profiles during the study varied significantly between individuals. In

order to maximise the chance of picking up the stress response, the peak was defined as

the maximum of the three post stress samples. The baseline on the study day was taken as the minimum of the first four samples. The difference between these two variables was defined as the stress response. The mean of the two afternoon samples during the diurnal curve served as a non-stressed baseline from which the stress response was also calculated where the data was available. See sections 3.3.2.1 and 3.3.2.2 for full explanation.

Heart rate and cortisol concentrations had a skewed distribution in this sample and were logarithmically transformed prior to analysis. Fisher-Yates normal scores were used to transform the stress responses where negative values prevented logarithmic transformation. Descriptive statistics are presented as mean (sd) (geometric where necessary), but medians and interquartile ranges are presented for variables transformed using normal scores.

The associations between size at birth and stress reactivity (cortisol and haemodynamic) were assessed by correlation and multiple linear regression controlling for gestation and other potential confounding factors. To allow comparison with previous data in the field, associations with resting blood pressure were assessed using the Dinamap data. In addition to the abstracted birth measurements, two others indices of fetal growth were examined: placental weight to birthweight ratio and ponderal index (birthweight/length^3). The association between cortisol concentration and blood pressure was also assessed. Significant gender differences were found in the blood pressure and cortisol responses to stress and thus all analysis was performed separately for the two sexes.

3.3 RESULTS

3.3.1 Basic characteristics of the participants

One hundred and four men and 80 women completed the study. One girl was found to be a significant outlier; she was born at 31 weeks gestation (all other participants were born at 36 weeks or later) and weighed only 1290g at birth. In addition, her blood pressure data were unusable as there were multiple interruptions in her Portapres trace. She was therefore excluded from all analyses. The mean age of the participants was 26.3yrs (sd 0.4). The men were approached first and thus, on average, were slightly younger when they were studied (mean difference 0.4 yrs (95% CI 0.3-0.5), $p<0.001$). Due to gender differences in the main outcome variables of the study and in the associations between size at birth and these variables, all data are presented for the sexes individually. The characteristics of the group at the time of the present study are detailed in Table 3.1. Forty-one women (52%) were using hormonal contraception and, of the remainder, 15 (44%) were in the follicular phase of the menstrual cycle.

Table 3.2 shows the characteristics of the participants at birth. Birthweight ranged from 1560-4710g in the men and 2110-4410g in the women. Corresponding ranges for length were 40.0-56.0cm and 44.0-53.5cm and head circumference were 29.0-39.5cm and 30.0-39.5cm. Birth measurements in this group were compared with those members of the cohort who did not take part in the study. Head circumference was 0.3cm (CI: 0.0-0.6, $p=0.03$) greater in the participants, but there were no other significant differences (Table 3.3). Two indices of socio-economic status in childhood were kindly made available for further comparison; father's occupational status and mother's educational attainment when the cohort was first assembled at eight years of age. These variables did not differ between participants and non-participants (Table 3.3).

Table 3.1 Characteristics of the participants at the time of the stress study.

	Men Mean (sd)	**Women** Mean (sd)
N	104	79
Age (yrs)	26.1 (0.4)	26.5 (0.3)
Anthropometry		
Height (cm)	179.7 (6.7)	164.1 (5.9)
Weight (kg)	82.5 (12.3)	67.8 (15.3)
BMI[a] (kg/m^2)	25.3 (1.1)	24.6 (1.2)
Waist[a] (cm)	87.2 (1.1)	78.2 (1.1)
WHR	0.85 (0.05)	0.77 (0.05)
Dinamap Blood Pressure		
Systolic BP (mmHg)	118.1 (10.8)	105.6 (10.5)
Diastolic BP[a] (mmHg)	61.1 (1.1)	57.8 (1.1)
Lifestyle variables		
Married (%)	27.9	53.2
Current smoker (%)	34.6	30.4
Alcohol >10U/wk (%)	43.3	20.3
University (%)	36.5	44.3
IRSD	1015 (82)	1021 (81)

[a]Geometric mean (sd)
BMI: body mass index, WHR: waist hip ratio, BP: blood pressure
IRSD: Index of Relative Socioeconomic Disadvantage based on postcode of residence; population mean: 1000

99

Table 3.2 Characteristics of the participants at birth.

	Male *Mean (sd)*	**Female** *Mean (sd)*
N	104	79
Gestation (weeks)	39.1 (1.4)	39.4 (1.3)
Birthweight (g)	3545 (576)	3279 (445)
Length (cm)	50.6 (2.5)	49.2 (1.9)
Head circumference (cm)	35.0 (1.6)	34.3 (1.5)
IRSD at birth[a]	1016 (72)	1022 (70)

IRSD: index of relative socioeconomic disadvantage based on parents' postcode at birth; population mean: 1000

Table 3.3 Comparison of birth characteristics of the participants with members of the cohort who did not participate.

	Participants *Mean (sd)*	**Non-participants** *Mean (sd)*	**p value for difference**
N	183	639	
Gestation (weeks)	39.2 (1.3)	39.1 (2.0)	0.3
Birthweight (g)	3430 (538)	3367 (559)	0.2
Length (cm)	50.0 (2.4)	49.7 (2.7)	0.2
Head circumference (cm)	34.7 (1.6)	34.4 (1.7)	0.03
IRSD at birth	1018 (71)	1016 (71)	0.7
Maternal education (%)			
No high school	8	8	
Completed high school	28	31	0.8
Tertiary education	25	24	
Father's occupation in lowest category[a] (%)	11	14	0.4

IRSD: index of relative socioeconomic disadvantage based on parents' postcode at birth; population mean: 1000
[a]Congalton status category D – unskilled, manual

3.3.2 Cortisol response to psychological stress

3.3.2.1 Descriptive statistics

Complete cortisol data from the stress study were available for 84 men and 60 women.
The remainder did not produce enough saliva for analysis of a variable number of
samples. Nine participants (6 male) missing four or more of the seven samples were
excluded from the analysis as no clear interpretation of their results could be made. Mean
salivary cortisol concentration over the course of the study is shown in Figure 3.3. Men
had higher cortisol concentrations throughout, and the difference became significant from
the Stroop sample onwards (Stroop and Mirror: $p<0.05$, Speech – Recovery 2: $p<0.001$).

Figure 3.3   Mean (sem) salivary cortisol concentration in men (n=98) and women (n=76) over the course of the stress study.
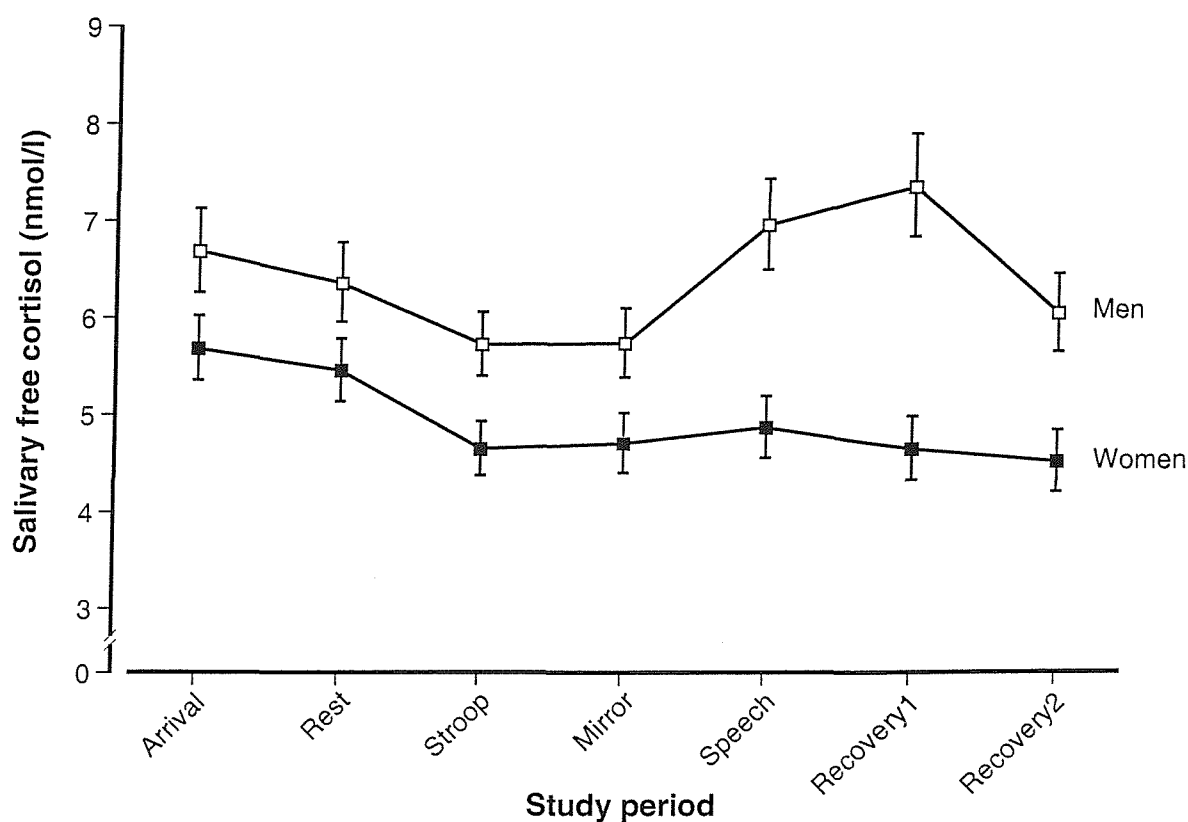
Recovery 1: 20 mins after the speech task, Recovery 2: 30 mins after the speech task

On average, cortisol concentration declined over the first three samples in both sexes and thereafter the profiles diverged, with men showing a definite stress response. In women, the response was less marked, but as cortisol concentrations would normally continue to decline over course of the afternoon, the attenuation of the drop after the first three samples suggests a small stress response. Individual profiles differed considerably which made choosing appropriate summary statistics difficult. Amongst the men, 51% showed a clear cortisol response following the stress tests, 28% had a double peak (high levels on arrival and elevation post stress, though often to a lesser degree) and 21% declined consistently from arrival. Corresponding figures for women were: 32%, 38% and 30%.

On the basis of the mean profile, the following variables were derived:

Pre-stress baseline: minimum of the first four samples

Post-stress peak: maximum of the last three samples

Study stress response: peak minus baseline

As arrival at the clinic and subsequent anthropometric measurement and fitting of the Portapres is likely to have been a significant stressor for some individuals, the cortisol concentration at the start of the study was also used as a marker of stress responsiveness. The mean cortisol concentration over the course of the study provided a measure of overall glucocorticoid exposure. Table 3.4 details the descriptive statistics for these variables.

In addition, participants were characterised as responders or non responders by several methods; inspection of the curves and, based on the study stress response, median split, top and bottom 40% and top and bottom tertiles.

3.3.2.2   Baseline afternoon salivary cortisol concentration

As described in the section 3.2.3, participants were asked to collect further salivary cortisol samples shortly after the stress study to provide a baseline away from the clinic environment from which to calculate the stress response. Unfortunately, the data obtained was incomplete and of poor quality, both in terms of the times the samples were taken and the length of time that had elapsed following the study. One hundred and twenty-four (71%; 69 men, 55 women) of the 174 subjects with useable salivary cortisol data from the stress study returned their samples; median time interval of 14 (IQR: 6-39) days. Overall, 94% of these samples had been taken within two hours of the requested times; 1200h and 1600h. Five men had collected their samples much later than requested and these values were therefore excluded. In addition, 10% of the assays registered as either acidic or alkaline, rendering the results unreliable.

Baseline afternoon salivary cortisol concentration was taken as the mean of the two samples (having excluded the late samples and the unreliable assays). In men, the afternoon baseline was significantly lower than the pre-stress baseline on the study day (3.9 vs 4.8 nmol/l; n=52, p=0.007) and there was a similar trend in women (3.4 vs 3.9; n=48, p=0.06). This value was therefore used to calculate two additional measures of stress responsiveness in those subjects for whom the data was available.

Arrival response: increment to the first sample on the day of the stress study

Maximum stress response: increment to the post-stress peak.

Table 3.5 details the descriptive statistics for these variables.

Table 3.4 Descriptive statistics for variables derived from the cortisol profiles.

Salivary cortisol concentration (nmol/l)	Men	Women	p value for sex difference
N	98	76	
Mean[a]	6.7 (1.8)	5.2 (1.7)	0.002
Arrival[a]	6.7 (1.9)	5.7 (1.7)	0.07
Pre-stress baseline[a]	4.9 (1.8)	4.3 (1.7)	0.1
Post-stress peak[a]	7.8 (2.0)	5.3 (1.8)	<0.001
Study stress response[b]	2.1 (0.3-5.1)	0.5 (0.0-1.5)	<0.001

[a]geometric mean (sd)
[b]median (IQR)

Table 3.5 Descriptive statistics for variables derived from the baseline data.

Salivary cortisol concentration (nmol/l)	Men	Women	p value for sex difference
N	52	48	
Afternoon baseline[a]	3.9 (1.8)	3.4 (1.5)	0.2
Arrival response[b]	3.0 (0.6-5.4)	1.1 (-0.3-3.5)	0.09
Maximum stress response[b]	2.1 (0.4-5.9)	0.9 (-0.2-2.4)	0.07

[a]geometic mean (sd)
[b]median (IQR)

3.3.2.3 Determinants of the cortisol stress response

In order to complete the study within the time available, it was necessary to do two studies per afternoon session. Subjects were therefore offered an appointment at either 1330h or 1530h. Initial cortisol concentrations tended to be higher in those men studied before 1500h, but there were no differences in the post stress samples or in the study stress response (Table 3.6). Cortisol concentration did not differ by appointment time in the female participants and there were also no significant differences in the stress responses calculated from the afternoon baseline (p=0.6 for all).

Table 3.6 Salivary cortisol concentration according to time of study.

Mean (sd) salivary cortisol (nmol/l)	Start of study		p
	Before 1500h	**After 1500h**	
Male (n)	46	52	
Arrival	7.5 (1.8)	6.0 (1.9)	0.1
Post-stress peak	7.6 (2.0)	7.7 (2.0)	0.9
Study stress response[a]	2.0 (0.1-4.7)	2.2 (0.5-5.4)	0.4
Female (n)	40	36	
Arrival	5.9 (1.5)	5.4 (1.8)	0.4
Post-stress peak	5.4 (1.8)	5.2 (1.8)	0.8
Study stress response[a]	0.5 (-0.1-1.3)	0.5 (0.0-1.5)	1.0

[a]median (IQR)

There were no differences in cortisol concentration or response dependent on which of the three investigators supervised the study (p>0.5 for all comparisons).

There were no significant associations between the anthropometric variables and the absolute cortisol concentrations in either sex. In men, the maximum stress response was positively correlated with central obesity (WHR: r=0.29, p=0.04), whilst in women there was a strong inverse relationship (WHR: r=-0.42, p=0.004) though this was somewhat influenced by outliers. In men, the study stress response tended to be lower in smokers (1.1 vs 2.5 nmol/l, p=0.1), but this was not mirrored in the maximum stress response (2.3 vs 1.9 nmol/l, p=0.7). No associations were found with alcohol consumption, marital status or physical activity in men or women.

There was trend towards a higher afternoon baseline cortisol concentrations in men from disadvantaged areas (r=-0.28, p=0.05), but the stress responses were not significantly correlated with IRSD. Educational attainment and job classification were not related to cortisol concentration or stress responses. Likewise, neither depression score nor the presence of a current major life stressor predicted cortisol concentrations. Menstrual cycle phase and hormonal contraceptive use did not influence the results in the female subjects.

In men, cortisol concentrations were higher in subjects who woke later on the morning of the study (mean salivary cortisol vs hour of waking: r=0.20, p=0.05), but the stress response was not influenced by time of waking. Subjects were asked whether they had been to work on the morning of the study and if they had experienced any difficulty getting to the clinic; neither of these variables were determinants of initial cortisol concentration or the cortisol response to stress. Performance during the Stroop (number of attempts and percentage correct) and mirror (number of circuits and errors) tasks was not related to the stress response in either sex.

3.3.2.4 Association between size at birth and the cortisol stress response

Univariate analysis revealed weak inverse relationships between salivary cortisol concentrations during the stress study and birthweight, length, head circumference and gestational age in men (Table 3.7). Cortisol concentrations were higher throughout the study in men who were smaller at birth, but the relationship was slightly stronger after stress. There was no association between size at birth and the magnitude of the stress response.

Table 3.7 Pearson correlation coefficients relating cortisol concentration to size at birth in men.

Salivary cortisol	Weight	Length	Head	PI	Gest[n]
Mean	-0.10	-0.17	-0.12	0.05	-0.19
Arrival	-0.08	-0.13	-0.07	0.02	-0.11
Pre-stress	-0.06	-0.18	-0.09	0.12	-0.16
Post-stress	-0.12	-0.17	-0.12	0.05	**-0.20[a]**
Study response	-0.06	-0.07	-0.03	0.01	-0.07
Baseline	-0.03	-0.02	0.02	0.03	-0.06
Arrival response	-0.12	-0.03	-0.11	-0.23	0.06
Maximum response	-0.12	-0.20	-0.13	0.02	0.00

PI: ponderal index
[a]$p < 0.05$

Relationships with the birth measurements were removed by adjustment for gestational age (for example post-stress peak: $r = 0.04$, $r = 0.02$, $r = 0.05$ for birthweight, length and head circumference respectively). In multiple linear regression analysis, the association between peak post stress cortisol concentration and gestational age was not altered by

including hour of waking and smoking status in the model. With each additional

completed week of gestation, cortisol fell 1.1-fold (95% CI: 1.0 to 1.2, p=0.05).

There were no significant linear associations between any of the salivary cortisol

parameters and size or maturity at birth in the female participants (Table 3.8).

Table 3.8 Pearson correlation coefficients relating cortisol concentration to size at birth in women.

Salivary cortisol	Weight	Length	Head	PI	Gest^n
Mean	-0.03	-0.05	0.03	0.02	0.01
Arrival	-0.02	-0.04	0.00	0.00	0.03
Pre-stress	-0.05	-0.05	-0.05	-0.03	0.07
Post-stress	-0.01	-0.04	0.07	0.03	-0.04
Study response	0.09	0.04	0.18	0.11	-0.15
Baseline	0.21	0.03	0.16	0.28	0.19
Arrival response	-0.02	0.03	-0.07	-0.09	0.00
Maximum response	-0.21	-0.19	0.00	-0.05	-0.02

PI: ponderal index

The cortisol variables were also carefully examined according to sex-specific quintiles of

the birth measurements and squared terms were included in the regression models to look

for quadratic relationships; none were found. In addition, no significant associations

between size at birth and cortisol responses to psychological stress emerged when the data

were analysed with the sexes combined. Logistic regression revealed no effects of size at

birth on responder status (data not shown).

3.3.3   Haemodynamic responses to psychological stress

3.3.3.1   Descriptive statistics

Haemodynamic data were unavailable for one male and three female participants due to technical failure of the Portapres. A further six men and three women had interrupted recordings, but as these did not occur during any of the marked periods their data was included. Excluding them did not significantly change the results of the analysis.

Blood pressure and heart rate rose during the tasks and dropped back towards resting levels between tasks and during recovery in both men and women (Figure 3.4). The speech task produced the greatest increment from baseline; blood pressure rose 39/21 (±15/7) mmHg while heart rate rose 13 (IQR 8-20) bpm. The haemodynamic variables were averaged across the three tasks to produce a reliable measure of stress reactivity for each individual. Mean systolic blood pressure did not differ in men and women at rest, between tasks or during recovery, but rose more in men during stress (mean difference 5 mmHg (95% CI: 2-8), p=0.003). Diastolic blood pressure did not differ significantly between the sexes at any point during the study. Women tended to have higher heart rates throughout (p=0.01 – 0.1), but stress responses were similar (p=0.6) (Table 3.9).

Blood pressure was also measured with a Dinamap at three time points in the study as detailed in Table 3.10. The Dinamap readings were significantly lower than the corresponding Portapres measurements and the two methods were not closely correlated (r=~0.50). Associations between size at birth and resting blood pressure were therefore based on Dinamap readings to be consistent with the literature in this field.

Figure 3.4   Systolic and diastolic blood pressure (upper panel) and heart rate (lower panel) in men (n=103) and women (n=76) over the course of the stress study.
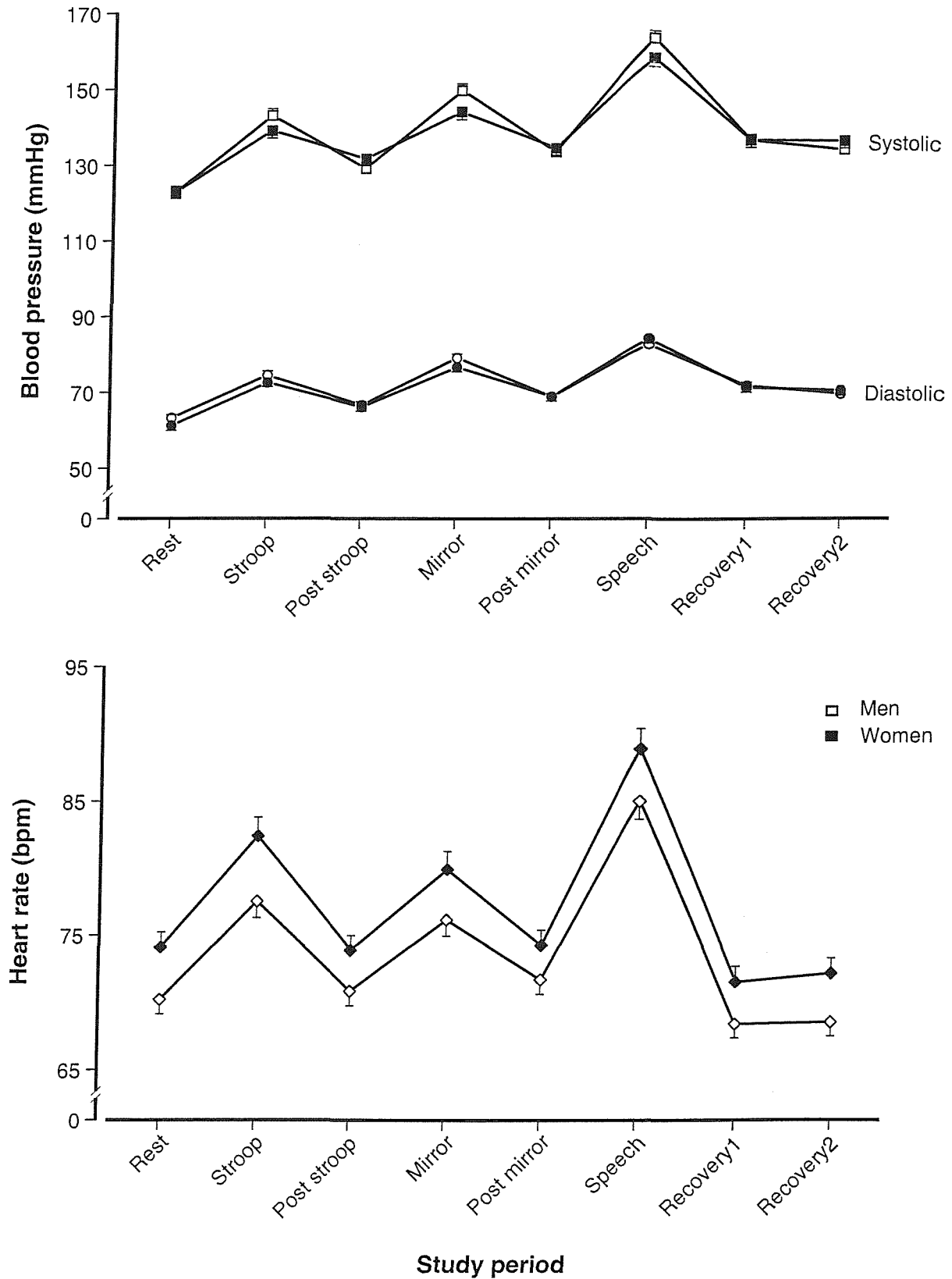
Data points represent 5 minute mean (sem) for each phase.
Recovery 1: 5-10 mins after speech, Recovery 2: 25-30 mins after speech.

Table 3.9 Haemodynamic variables measured with the Portapres in men and women at rest, during stress and at the end of the study.

	Men *Mean (sd)*	Women *Mean (sd)*	p value for sex difference
N	103	76	
Rest			
Systolic BP (mmHg)	123 (15)	123 (15)	1.0
Diastolic BP (mmHg)	63 (9)	61 (10)	0.2
Heart Rate[a] (bpm)	70 (1.2)	74 (1.1)	0.02
Stress (average)			
Systolic BP (mmHg)	152 (17)	147 (18)	0.06
Diastolic BP (mmHg)	79 (10)	78 (10)	0.5
Heart Rate[a] (bpm)	80 (1.2)	84 (1.1)	0.03
Stress response			
Systolic BP (mmHg)	29 (12)	24 (11)	0.003
Diastolic BP (mmHg)	16 (5)	17 (5)	0.2
Heart Rate[b] (bpm)	8 (5-13)	10 (5-13)	0.6
Recovery			
Systolic BP (mmHg)	134 (15)	136 (16)	0.3
Diastolic BP (mmHg)	69 (9)	71 (12)	0.5
Heart Rate[a] (bpm)	68 (1.2)	72 (1.2)	0.02

[a]Geometric mean (sd)
[b]Median (IQR)
BP: blood pressure
Stress (average): mean during the three tasks
Stress response: Increment from rest to the stress average

Table 3.10 Haemodynamic variables measured with the Dinamap in men and women at rest, after the speech and at the end of the study.

	Men *Mean (sd)*	**Women** *Mean (sd)*	**p value for sex difference**
N	95	79	
Rest			
Systolic BP (mmHg)	118 (11)	106 (10)	<0.001
Diastolic BP[a] (mmHg)	61 (1.0)	58 (1.1)	0.004
Heart Rate[a] (bpm)	64 (1.2)	68 (1.2)	0.02
Post speech			
Systolic BP (mmHg)	127 (10)	122 (11)	<0.001
Diastolic BP[a] (mmHg)	67 (1.1)	63 (1.1)	<0.001
Heart Rate[a] (bpm)	66 (1.2)	68 (1.1)	0.2
Recovery			
Systolic BP (mmHg)	122 (11)	108 (11)	<0.001
Diastolic BP[a] (mmHg)	64 (1.1)	60 (1.1)	0.004
Heart Rate[a] (bpm)	65 (1.2)	70 (1.1)	<0.001

[a]Geometric mean (sd)
BP: blood pressure

3.3.3.2 Determinants of the haemodynamic stress response

Stress responses differed between the three investigators (Table 3.11), but were not influenced by the time at which the study commenced.

Table 3.11   Comparison of stress responses between investigators.

Investigator	N	Systolic response (mmHg)	Diastolic response (mmHg)	Heart rate response (bpm)
Men				
1	64	30 (12)	16 (5)	8 (5-11)
2	23	32 (11)	17 (6)	10 (5-15)
3	16	23 (9)[a]	12 (5)[a]	9 (4- 13)
Women				
1	67	23 (10)	16 (5)	10 (5-13)
2	12	29 (12)	19 (5)[a]	11 (5-16)

[a]significantly different from investigator 1, p<0.05

Resting blood pressure did not predict stress responses in either sex. In general, blood pressure reactivity was not related to current size or obesity, though diastolic responses fell with increasing WHR in women (r=-0.25, p=0.03). In men, the heart rate response to stress was inversely related to obesity (BMI: r=-0.20, p=0.04) and similar trends were seen in women (WHR: r=-0.23, p=0.04).

The haemodynamic changes were not related to smoking status in men. Systolic blood pressure responses were reduced in female smokers (20 vs 26 mmHg, p=0.01), but diastolic responses did not differ (16 vs 17 mmHg, p=0.3). Heart rate response also tended

to be lower in women who smoked (7 vs 10 bpm, p=0.05). Alcohol consumption, marital status and physical activity did not predict stress responsiveness in either sex.

Various measures of socioeconomic status were examined. Level of educational attainment and job classification did not predict blood pressure or heart rate responses to stress. In men, stress responses were positively related to current IRSD (SBP: r=0.18, p=0.07; DBP r=0.28, p=0.005). There were no significant associations with current IRSD in women, but IRSD at birth predicted stress responses; women born in deprived areas had lower responses (SBP: r=0.24, p=0.04; DBP r=0.20, p=0.09; HR r=0.24, p=0.04).

Women with higher scores on the CES-Depression scale had lower stress responses (SBP: r=-0.25, p=0.04; DBP: r=-0.23, p=0.05; HR r=-0.22, p=0.07) and there was a similar trend for systolic reactivity in men (r=-0.18, p=0.08). Menstrual cycle phase did not influence cardiovascular reactivity. Blood pressure responses to stress did not differ in contraceptive users (systolic 25 vs 24 mmHg, p=0.7; diastolic 17 vs 16 mmHg, p=0.2), but heart rate responses were higher (11 vs 6 bpm, p=0.002). In general, performance during the tasks did not determine stress reactivity, but there was a positive correlation between the number of mirror circuits completed and the heart rate response in men (rho=0.25, p=0.01) and the number of Stroop attempts was related to the blood pressure responses in women (SBP: rho=0.28, p=0.01; DBP: rho=0.23, p=0.05).

3.3.3.3 Association between size at birth and resting blood pressure

There were no significant relationships between the Dinamap resting systolic blood pressure and birthweight in either men or women (men: β= -1.2 *(95% CI: -5.5 to 3.0)* mmHg/kg, p=0.6; women β= -2.5 *(-7.8 to 2.8)* mmHg/kg, p=0.4). Correcting for current weight strengthened the association in women (β=-4.2 (-9.1 to 0.7) mmHg, p=0.09), but not in men. These results were compared with data collected from a larger sample of the cohort at 20 years of age; the relationships in the current study were weaker throughout, but the direction of effect was the same as in the previous investigation (Table 3.12) (210). There were no significant associations between resting diastolic blood pressure and size at birth in either sex, as in the previous investigation.

Table 3.12 Correlation coefficients relating birth variables to Dinamap resting systolic blood pressure in comparison with similar data collected at age 20.

Systolic BP	MEN		WOMEN	
	Age 20[a] (n=297)	Age 26 (n=95)	Age 20[a] (n=287)	Age 26 (n=79)
Birthweight	-0.14 -0.07	-0.06 -0.03	-0.22 -0.16	-0.11 -0.11
Length	-0.11 -0.03	-0.04 0.01	-0.21 -0.16	-0.17 -0.17
PI	-0.13 -0.10	-0.05 -0.06	-0.15 -0.11	0.05 0.04
PL:BWT	0.13 0.09	0.14 0.10	0.14 0.10	0.13 0.14

Each cell contains the Pearson correlation coefficient relating the two variables and beneath it the partial correlation coefficient corrected for gestational age
[a]Data reproduced with permission (Dr V Moore)
BP: blood pressure
PI: ponderal index
PL:BWT: placental weight to birthweight ratio

3.3.3.4 Association between size at birth and the haemodynamic stress response

Relationships between birth measurements and resting systolic blood pressure measured with the Portapres were similar to those seen with the Dinamap (men: β=-1.0 (-6.2 to 4.2) mmHg/kg, p=0.7; women: β=-3.2 (-10.9 to 4.5) mmHg/kg, p=0.4). However, during stress, significant inverse associations between average systolic blood pressure and size at birth emerged in female subjects (β=-11.9 (-20.5 to -3.3) mmHg/kg, p=0.007) (Table 3.13). There were similar trends with diastolic blood pressure and heart rate, though these did not reach statistical significance (data not shown). There were no relationships between stressed systolic blood pressure and size at birth in men (β=1.4 (-4.5 to 7.3) mmHg/kg, p=0.6) (Table 3.13).

Table 3.13 Pearson correlation coefficients relating size at birth to systolic blood pressure at rest and during psychological stress.

	MEN (n=103)		WOMEN (n=76)	
	Resting SBP	Stressed SBP	Resting SBP	Stressed SBP
Birthweight	-0.04	0.05	-0.10	**-0.31**[a]
Length	-0.10	0.00	-0.06	**-0.24**[b]
Head circ	-0.02	0.01	-0.13	**-0.34**[a]
PI	0.09	0.05	-0.03	-0.12
PL:BWT	0.16	0.15	-0.13	0.06
Gestation	-0.12	-0.05	0.02	-0.07

SBP: systolic blood pressure
PI: ponderal index
PL:BWT: placental weight to birthweight ratio
[a]p<0.01; [b]p<0.05

Strong inverse correlations between birthweight, length and head circumference and all aspects of the haemodynamic response to psychological stress were observed in women (Table 3.14). There were also weaker inverse relationships with ponderal index and positive associations with placental weight to birthweight ratio. None of these associations were influenced by gestational age. A 1kg increase in birthweight was associated with an 8.7 mmHg (3.6 to 13.8, p=0.001) reduction in systolic and a 4.1 mmHg (1.6 to 6.6, p=0.002) reduction in diastolic response to stress. Corresponding figures for a one centimetre increase in birthlength were systolic: 1.7 mmHg (0.5 to 3.0), p=0.006 and diastolic: 0.8 mmHg (0.2 to1.4), p=0.006 and for a one centimetre increase in head circumference were systolic: 2.6 mmHg (1.1 to 4.0), p=0.001 and diastolic: 1.4 mmHg (0.7 to 2.1), p<0.001.

Table 3.14   Pearson correlation coefficients relating size at birth to haemodynamic responses to psychological stress.

	MEN (n=103)			WOMEN (n=76)		
	SBP response	DBP response	HR response	SBP response	DBP response	HR response
Birthweight	0.12	0.06	0.01	-0.37[a]	-0.36[a]	-0.31[a]
Length	0.14	0.11	-0.01	-0.31[a]	-0.31[a]	-0.23
Head circ	0.04	-0.03	0.04	-0.38[b]	-0.42[b]	-0.27[c]
PI	-0.05	-0.09	0.01	-0.16	-0.15	-0.16
PL:BWT	0.02	0.04	-0.01	0.30[c]	0.19	0.05
Gestation	0.08	0.10	-0.16	-0.14	-0.01	-0.01

SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate
PI: ponderal index
PL:BWT: placental weight to birthweight ratio
[a]p<0.01; [b]p<0.001; [c]p<0.05

In contrast to the women, there were no associations between birth size and the haemodynamic response to stress in men (Table 3.14). The difference in the findings in the two sexes was statistically significant (for example, p=0.001 for the interaction term in birthweight systolic response model).

Table 3.15 shows the average blood pressure and heart rate stress response according to birthweight tertile in women. The average systolic response to stress in the bottom tertile (<3100g) was similar to the mean response in the men (29mmHg) whereas women who were heavier at birth showed reduced stress responses.

Table 3.15   Mean blood pressure and heart rate responses to stress according to birthweight tertile in women.

Birthweight tertile	N	SBP response	DBP response	HR response[a]
<3100	27	29	19	12
<3470	25	23	16	10
>3470	24	20	15	6
Total	76	24	17	10
SD/IQR		11	5	5-13
p[b]		**0.001**	**0.002**	**0.006**
p[c]		**0.02**	**0.03**	**0.02**

SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate
[a]Median
[b]based on continuous analysis
[c]corrected for investigator, smoking status, CES-D and IRSD at birth

In multiple linear regression analysis, investigator, smoking status, CES-D score and IRSD at birth were included in the model as potential confounders. Birthweight was the only variable that remained a significant predictor of stress response (Table 3.16). The model explained 24% of the variance in the systolic response, 20% of the variance in the diastolic response and 18% of the variance in the heart rate response to stress.

Table 3.16   Multiple linear regression analysis to determine predictors of the haemodynamic stress response.

	Stress Response					
	Systolic		Diastolic		Heart rate	
	β	p	β	p	β^a	p
Birthweight (kg)	-6.5	0.02	-3.1	0.03	-0.6	0.02
Investigator[b]	-4.9	0.1	-3.1	0.05	-0.2	0.5
Smoker	-3.2	0.2	0.0	1.0	-0.1	0.8
CES-D	-1.2	0.3	-0.7	0.2	-0.1	0.4
IRSD	0.02	0.2	0.01	0.3	0.00	0.1

[a]change in heart rate standard deviation score per unit of predictor variable
[b]female studies were carried out by one of 2 investigators
CES-D: Centre for Epidemiologic Studies depression score
IRSD: Index of Relative Socioeconomic Disadvantage based on postcode of residence at birth

3.3.4   Task performance and psychological response to stress

Objective assessment of performance was possible for the Stroop test (number of attempts and percentage correct) and the mirror drawing (number of circuits and errors). There were no gender differences in the Stroop performance, but women completed more circuits of the star (7 vs 6, p=0.04) at the expense of a greater number of errors (17 vs 9, p=0.002) while mirror-drawing. There were no significant associations between size at birth and task performance in the men, but, in women, head circumference positively correlated with the number of Stroop attempts (rho=0.23, p=0.04) and was inversely related to the number of mirror errors (rho=-0.25, p=0.03).

Immediately after each task, subjects were asked to rate how difficult they found the task, how involved they were in it, their performance, the degree of control they had over the task and how stressed and relaxed they felt during the task (Appendix C). Scores for each of these aspects were averaged over the three tasks and, in addition, a summary score for the impact of each task individually was generated. There were no significant gender differences in how the tasks were rated and, of the individual aspects, only average performance rating differed: women rated their performance lower than men (4.6 vs 4.2 (scale 1-7, higher score = worse performance), p=0.007). Subjective stress ratings at the end of the rest and recovery periods were assessed using a slightly different scale (Appendix C). To assess the psychological impact of the stressors the two items that were present in both scales (stressed and relaxed) were averaged for the rest period, across the three tasks and for the recovery period. Subjective stress ratings (scale 1-7) rose significantly from 2.0 at rest to 4.4 during the tasks and then fell to 1.8 by the end of recovery (p<0.001 for all differences). There were no correlations between size at birth and any of the task impact scores in univariate analysis.

3.3.5 Relationship between different components of the stress response

Resting blood pressure was positively correlated with baseline cortisol in men (SBP: r=0.32, p=0.002; DBP: r=0.23, p=0.03), but not in women (SBP: r=0.18; DBP: r=-0.03). Cortisol and blood pressure responses to psychological stress were also positively correlated (Table 3.17).

Table 3.17 Pearson correlation coefficients relating blood pressure and cortisol responses to stress.

Salivary cortisol	MEN		WOMEN	
	Systolic response	Diastolic response	Systolic response	Diastolic response
Post-stress peak	0.22[a]	0.15	0.07	0.26[a]
Study stress response	0.24[a]	0.22[a]	0.19	0.23
Maximum stress response	0.17	0.18	0.30[a]	0.40[b]

[a]$p<0.05$, [b]$p<0.01$

Haemodynamic responses were not related to subjective stress ratings in men. In women, the systolic response was inversely correlated with the rating of the speech task (r=-0.31, p=0.008), suggesting somewhat paradoxically that those individuals who found the speech task most stressful had the smallest response.

Stress rating at the end of the rest period was a significant predictor of the post-stress peak salivary cortisol concentration in men (r=0.27, p=0.01), but it was not significantly correlated with the stress response. There were no other significant associations between subjective task impact and the cortisol responses.

3.4   DISCUSSION

This study has examined the relationships between size at birth and two aspects of the

physiological response to a psychological stress test in a group of young men and women.

The individuals who participated in the study were a recruited as a random sample from an

established cohort, stratified by birthweight to ensure that a representative group was

studied. Apart from head circumference, birth measurements did not differ between the

participants and the others members of the cohort and several markers of socio-economic

status were also similar. The analysis presented is based on internal comparisons within

the study group and thus, unless relationships between fetal growth and stress

responsiveness are systematically different from those in non-participants, the results are

unlikely to be influenced by selection bias.

3.4.1   Fetal programming of the adrenocortical stress response

The primary aim of the study was to explore the hypothesis that HPAA responses to

psychological stress may be programmed antenatally in humans. An inverse relationship

between birthweight and fasting 0900h cortisol concentrations has been found in a number

of studies (110, 111), but more detailed examination of basal glucocorticoid secretion in

one of these cohorts found no such relationship (115). One possible explanation for this

dichotomy is that the combination of fasting, venepuncture and the unfamiliar clinic

setting in which the samples were taken for the initial studies constitutes a significant

stressor and thus that the 0900h cortisol concentrations partly reflect stress responsiveness.

The impact of size at birth on adrenocortical stress responses has not been examined

systematically in humans previously.

The principal psychological stimuli to the HPAA are helplessness, unpredictability, lack of control and anticipation of negative consequences (57). It is well known that producing a reliable adrenocortical response to psychological stress is difficult to achieve in the laboratory setting. The TSST appears to be the most consistent method reported in the literature, reproducibly provoking a two to four-fold elevation in salivary cortisol concentration above baseline and achieving a responder rate of 70-80% (204). This test was not used in the current study for two reasons. Firstly, as the protocol requires participants to perform a ten minute speech and mental arithmetic task while standing in front of an audience, our collaborators in Adelaide were concerned that the experience might deter members of the cohort from participating in future studies; a risk they were not willing to take. Secondly, the method has not been validated for assessing cardiovascular reactivity. It is likely that recording blood pressure with the subject standing would be a less reliable means of assessing haemodynamic stress responsiveness, as postural and motor factors would also be involved (213).

The protocol adopted has been used previously for assessment of both haemodynamic and adrenocortical stress responses (206). Various measures were added to increase the stimulus to the HPAA, including the arrival of the second investigator prior to the speech and exposure of the two-way mirror. The stressors produced a clear salivary cortisol response in men, but, in women, attenuation of the diurnal fall was the only evidence of a response. This gender difference is entirely consistent with published data, including that from the Trier group who suggest it may be due to enhanced stress-induced hypothalamic drive in men; ACTH concentrations are also higher following the TSST in men and yet there are no gender differences in the ACTH response to CRH, ruling out a difference in pituitary sensitivity (214).

Salivary cortisol concentrations were higher throughout the study in men who were small at birth, although these differences were largely due to prematurity rather than growth restriction. There were no associations between size at birth and salivary cortisol concentrations in women. Fasting 0900h plasma cortisol concentration was measured in a previous study in this cohort. The sub-sample was of similar size (n=165) to the current study and about a third of the subjects participated in both studies (n=65). In the former group, an inverse association between birthweight and cortisol was found in men and women, although this did not reach statistical significance in either sex. The current study assessed salivary free cortisol rather than total plasma cortisol and was performed at a different part of the diurnal cycle of HPAA activity, making comparison of the results in the two studies difficult.

There was no evidence from the present analysis that salivary cortisol responses to psychological stress are related to size at birth. Several practical problems were encountered that may have influenced the quality of the data. A significant number of the participants (19% men and 24% women) did not produce enough saliva for a complete cortisol series, despite keeping the Salivette in their mouths for two minutes. In other studies, incomplete data were excluded from the analysis (206), but restricting the data to only those subjects with a full series would have severely limited the power of the present study. Hence, the data presented are based on at least four samples, two of which were in the post-stress period. Repeating the analysis on complete data sets did not produce substantively different results, but trying to ensure adequate saliva samples should be imperative in future studies of this nature.

It was clearly apparent from examining the salivary cortisol profiles that patterns of response differed markedly between individuals which made summarising the data difficult. As cortisol concentrations continued to decline after the end of the rest period in the majority of participants and the response to stress was seen, if at all, in the recovery period, the baseline was chosen as the minimum of the first four samples and the peak as the maximum of the last three. This approach, which has been reported elsewhere (205), generated the largest cortisol increment following the stressors for each individual, ignoring the time course of the response. Increments between specific time points and the area under the curve were also examined, but the associations reported did not differ. Several studies assign responder and non-responder status to their participants and analyse the data accordingly. Attempts were made to do this in the current data set by several means, but in no case did the split add to the results generated by continuous analysis of the data.

It was anticipated that the whole session would constitute a stressful experience and this was confirmed in those individuals who took saliva samples during a normal working day following the study. Despite the uncontrolled circumstances in which these samples were taken, the mean afternoon cortisol concentration was significantly lower than the nadir of the early samples on the study day. The rest period in this study was only twenty minutes long. It is possible that those individuals with a pronounced arrival response did not have sufficient time to recover; negative feedback rendering them refractory to the stressors. Ensuring that the participants were fully rested and defining an accurate baseline in the full sample may have increased the chances of finding associations with size at birth.

One of the prerequisites for performing the study in this cohort was that it should be non-invasive and thus only salivary cortisol concentrations could be measured. Blood sampling for ACTH, total cortisol, catecholamines and other mediators of the stress response, such as interleukins, would have allowed a more detailed examination of the associations between size at birth and the stress system and should be included in future studies if possible.

The lack of association between size at birth and HPAA stress responsiveness was disappointing given the wealth of animal data supporting the hypothesis that these responses may be programmed in early life (see section 1.4). Examining the reactivity of the entire HPAA using a central stimulus has clear theoretical advantages over pharmacological stimulation with either CRH or ACTH in mimicking real life fluctuations in glucocorticoid exposure. The psychological stressor used in the present study may not have provoked a response of sufficient magnitude or reliability to reveal antenatal influences, particularly in women. Further studies are therefore needed using stronger stimuli such as the TSST, accepting that assessment of blood pressure reactivity may be less reliable using this method. Alternatively, physiological stress tests may generate responses with less interindividual variability, increasing the power to pick up subtle differences associated with size at birth. Examples of such tests include protein-rich meals and the recently developed carbon dioxide breath test (215, 216).

3.4.2   Fetal programming of haemodynamic stress responses

Whilst this study was principally set up to explore relationships between adrenocortical

responses to psychological stress and markers of fetal growth, an assessment of the impact

of size at birth on haemodynamic responses was also possible. The pattern and magnitude

of the haemodynamic responses presented in this chapter are consistent with published

data (206). In the non-stressed state, there were no relationships between size at birth and

systolic or diastolic blood pressure. However, following a mild psychological stress,

women who were small at birth showed greater increases in both systolic and diastolic

blood pressure and heart rate. These findings provide the first human evidence that

cardiovascular reactivity may be programmed in utero.

The inverse relationship between birthweight and blood pressure was first published in

1986 and has been one of the most frequently reported and robust features of the 'fetal

origins' hypothesis in subsequent epidemiological studies (12). The influence of size at

birth on resting blood pressure has been studied twice previously in the cohort from which

the present group of participants was drawn. At eight years of age, there were no

significant associations between birth measurements and either systolic or diastolic blood

pressure in the cohort as a whole. However, inverse relationships with birthweight were

strengthened by including placental weight in the model; the highest blood pressures were

seen in low birthweight children with large placentas, a marker of fetal growth retardation

(209). At the age of twenty, 297 men and 287 women were studied independently.

Individuals who were small at birth had higher systolic blood pressures, particularly when

current weight was taken into account. These findings were stronger in women and

associations were also found with thinness at birth and low birthweight to placental weight

ratio. Further analysis showed that individuals with relatively low birthweight had greater

increases in blood pressure between the two studies (210). These earlier studies suggested that this would be useful group in which to examine the mechanisms underlying the early origins of raised blood pressure.

In the present study, at 26 years of age, associations between size at birth and resting blood pressure recorded with a Dinamap were in the same direction as those reported previously, but they were all were weaker and failed to achieve statistical significance, even after correction for current weight. This was largely due to the smaller numbers in the current study (183 vs 584), though it is possible that protocol differences may also have had an influence. Resting blood pressure is traditionally measured after a subject has been seated for five minutes (the case at age twenty), but in the current study participants had been sitting for over twenty minutes before their blood pressure was recorded. In light of the stress response-birthweight relationships reported in this chapter, it is possible that the findings were less marked as the subjects were truly rested.

The current study is the first to address the question of whether haemodynamic responses to psychological stress may be programmed in utero in humans. The strong inverse relationship between cardiovascular reactivity and size at birth in women suggests a possible mechanism by which fetal programming of hypertension may occur. The 'reactivity hypothesis' proposes that exaggerated cardiovascular responses to stress throughout life may ultimately lead to sustained hypertension through vascular remodelling and altered autoregulation and there is now a reasonable body of prospective data to support this hypothesis (201). The association between birthweight and resting blood pressure is generally stronger in older individuals and increased cardiovascular

reactivity in youth may be a marker of those low birthweight individuals more likely to go on to develop hypertension.

Stressed blood pressure has been related to birthweight in one previous study. This was a twin study set up to examine the role of genetic factors in birthweight-blood pressure associations. Blood pressure was measured with a Dinamap six times at rest and during reaction time and mental arithmetic tasks and the mean blood pressure for each phase was calculated. In the whole group (n=228, 61 monozygotic and 53 dizygotic twin pairs), inverse associations between recalled birthweight and systolic blood pressure were stronger during stress than at rest and became significant after adjustment for gender and current weight (217). These results mirror the findings of the current study and had stress responses been derived, the associations may have been stronger.

The results in the present study support the animal evidence that prenatal insults may alter the blood pressure response to stress. Tonkiss and colleagues found that prenatally-malnourished rats had greater increases in systolic and diastolic pressure in response to a recognised olfactory stressor than controls (202). These results led the authors to suggest that the blood pressures measured by indirect tailcuff plethysmography in the original protein-restriction models may have been stress responses (34). A recent report documents elevated pressor responses to restraint in the offspring of rats fed a high fat diet during pregnancy. However, corticosterone responses did not differ from controls, demonstrating a dissociation between the haemodynamic and adrenocortical stress response as found in the present study (218). In a model using unilateral uterine artery ligation to mimic placental insufficiency in rats, Jansson and colleagues found no correlation between birthweight and either mean arterial blood pressure or responses to moderate noise stress

in the offspring, though no control animals were included in their analysis. However, they found that sympathetic nervous system activity was inversely associated with birthweight in the females which is interesting in light of the results of the current study (40).

The mechanisms underlying cardiovascular reactivity during psychological stress are not well understood, but are likely to involve central autonomic output and adrenoreceptor density and sensitivity. There have been some preliminary studies reporting associations between heart rate reactivity and β-receptor function (213). Detailed haemodynamic assessment has revealed two distinct patterns of reactivity, producing similar blood pressure and heart rate responses. Cardiac responders show evidence of increased cardiac output, while vascular responders have increased total peripheral resistance and reduced cardiac output (219). These two patterns fall neatly into a predominately β-adrenoreceptor/adrenaline or an α-adrenoreceptor/noradrenaline response. There is evidence that cardiac reactors have a greater plasma adrenaline response to psychological stress and they have also recently been found to have raised 24hr urinary adrenaline concentrations which lends encouragement to the use of laboratory stress tests as markers of 'real life' responses (220). Similar alterations in noradrenaline concentrations have not been found in vascular responders. This is not necessarily surprising given that noradrenalaine is principally released as a neurotransmitter rather than directly into the circulation.

A number of animal studies have provided support for the hypothesis that the autonomic nervous system may be programmed in early life. Protein restriction through pregnancy and lactation resulted in increased circulating adrenaline and noradrenaline concentrations in male offspring at three months of age and also increased the expression of β_1 and β_3

receptors (123). However, another group found no differences in catecholamine concentrations when maternal food intake was limited globally to 50% normal (85). Weinstock and colleagues showed that prenatal stress did not alter resting catecholamine concentrations, but induced a greater rise in noradrenaline concentration in response to novelty and footshock than in control rats (124). Prenatal dexamethasone exposure was associated with increased resting plasma catecholamine concentrations in male rats, though due to the small numbers in this experiment the results did not reach statistical significance (125). In an ovine model, placental restriction increased circulating catecholamine concentrations in late gestation and it has been suggested that chronic hypoxia may be a stimulus to sympathetic hyperinnervation (127). Haemodynamic measurements were not made in any of these studies.

Human data examining early life influences on sympathoadrenal function are scanty. It is well recognised that growth-retarded fetuses have increased heart rates and raised catecholamine concentrations in cord blood (128). In one study, small-for-gestational age new-borns had increased heart rate and reduced heart rate variability when compared with controls during sleep (129). Inverse associations between resting pulse rate and birthweight have been reported in childhood and later in life and the possibility of autonomic nervous system programming has been inferred, though clearly this data needs to be backed up with more specific testing (130, 131). A recent case control study found that twelve year old children born small for gestational age had elevated circulating adrenaline, but similar noradrenaline concentrations (132).

The observation that associations between stress-induced increases in systolic and diastolic blood pressure and size at birth were confined to the women was unexpected, but

may be an important finding. Gender differences in fetal programming are frequently found in animal models (see sections 1.2 and 1.4) although they remain largely unexplained. In a recent example, rats dams were fed a lard-rich diet throughout pregnancy and lactation to mimic the high fat Western diet and blood pressure was assessed for seven days by radiotelemetry, on three occasions over the first year of life. Both systolic and diastolic blood pressure became progressively elevated in females compared with their controls while no differences were found in males. This was despite similar impairments in endothelium-dependent vasorelaxation in both exposed groups and serves to highlight the complexity of the mechanisms underlying these programming phenomena. The authors also noted that female offspring of lard-fed dams were less active than controls, but did not differ in terms of heart rate, hypothesising that this might reflect elevated basal sympathetic activity (36).

Many human studies concentrate on just one sex or pool data from both, making comparisons impossible. In a recent systematic review of the relationship between birthweight and blood pressure in humans, only fifteen of the eighty studies compared men and women. One reported a gender difference in the direction of the effect, though the relationship was not significant in either sex and five found a significant inverse association in only one sex (12). In stress studies, women are generally less responsive than men (221). While the findings in this study are consistent with this, they also suggest that the effect of low birthweight is to abrogate the gender differences in systolic blood pressure responses to stress. One possibility is that the mechanisms in women that constrain the activation of the sympathetic nervous system in response to stress are reduced in women who were small at birth.

Size at birth was not associated with emotional responses to stress in either sex, though the study was not designed to examine this question in any detail. The correlation between crude measures of performance in psychomotor tasks and head circumference at birth in women is in line with data suggesting that poor brain growth in utero has a long term influence on cognitive and motor ability (222). Salivary cortisol concentration was related to blood pressure in line with studies discussed in detail in sections 1.4.2.3 and 2.4.2.

In summary, the observations in this study do not support the hypothesis that stress reactivity of the HPAA may be programmed antenatally in humans. The study highlighted a number of the methodological problems of attempting to investigate this question and future studies would need to take these issues into account in order to improve the quality of the data. However, the study did identify a strong relationship between reduced fetal growth, as indicated by birth measurements, and exaggerated haemodynamic responses to psychological stress in young women. Despite the clear gender difference in this relationship, this novel finding suggests a potentially important mechanism underlying the fetal programming of hypertension in humans.

3.5 SUMMARY

1) In a group of young men and women size at birth was not associated with

   - Baseline salivary cortisol concentration

   - Salivary cortisol responses to psychological stress

2) Resting blood pressure and heart rate were not related to size at birth in either sex.

3) Cardiovascular responses to psychological stress were greater in women who were smaller at birth, but size at birth was not a determinant of stress responsivity in men.

CHAPTER 4

Conclusions and future directions

Advances in medical science occur through a variety of means, but observational studies within human populations frequently provide ideas from which other disciplines take their lead. Hence, studies linking size at birth with cardiovascular disease and its risk factors were received with great interest, if not a little scepticism, in the late 1980s and early 1990s. Since then, groups around the world have furthered the epidemiological understanding of this phenomenon and scientists, working principally in animal models, have started to unravel the underlying mechanisms. The 'fetal origins' hypothesis has stood up to ten years of investigation and looks likely to be the inspiration behind an increasing number of publications over the decades to come. In essence, exposure to a suboptimal antenatal environment appears to have damaging consequences that persist throughout the lifecourse of the offspring, via differential programming of structure and physiology.

At the time the work described in this thesis was being planned, some recently published human studies had added weight to animal data showing that the HPAA could be programmed by prenatal events. Low birthweight individuals had higher fasting morning cortisol concentrations in three populations and, in a follow up study, this was associated with exaggerated adrenal responses to ACTH stimulation (111, 117). As raised cortisol concentrations were also related to the classical risk factors for cardiovascular disease in these studies, programmed increases in HPAA activity seemed a plausible link between reduced fetal growth and cardiovascular disease in later life (110).

The research in this thesis was undertaken to explore the nature of the HPAA abnormality in low birthweight individuals in greater detail, in particular to determine whether the axis is upregulated above the level of the adrenal gland. Exaggerated ACTH responsiveness can reflect adrenal hypertrophy resulting from increased central drive, as seen in patients with Cushing's disease and major depression (223) and antenatal insults in animals have been shown to alter hypothalamic CRH content and hippocampal corticosteroid receptor expression, supporting the idea that abnormalities in HPAA activity may be programmed centrally (224).

In the first study (Chapter 2), three tests of HPAA activity were compared in a group of low and high birthweight individuals. The tests were chosen to examine pituitary responsiveness and hypothalamic drive. The results do not support the previous data, and showed no evidence of upregulation of the HPAA in men who were small at birth. In fact, in the one test that differentiated between the two groups, the dexamethasone-suppressed CRH test, the axis appeared to be less active in the low birthweight group. In retrospect, these contradictory findings may reflect the fact that there was no association between fasting morning cortisol and birthweight in this cohort, data which were unavailable at the start of the study. Thus, the study did not answer the question it was designed to address; as low birthweight individuals in this population were not hypercortisolaemic, the negative results do not rule out hypothalamic or pituitary abnormalities underlying the findings in previous studies.

Over the last few years, as other populations have been studied, it has become apparent that the clear inverse relationship between birthweight and cortisol found in the initial investigations is not ubiquitous. Birthweight is a crude marker of antenatal events and a

one-off cortisol measurement is a rather poor and non-specific means of assessing HPAA activity, heavily influenced by the conditions in which it is taken. Thus, it is perhaps surprising that robust associations between these measures have been found in any study. Future work needs to concentrate on antenatal exposures rather that size at birth, as highlighted in a group of young Scottish adults who have become a valuable resource for investigating the impact of extreme dietary manipulation in human pregnancy. Their mothers were advised to eat a high protein (1lb of red meat per day), low carbohydrate diet. In this cohort, there was no association between birthweight and fasting morning cortisol, but higher meat/fish intake in late pregnancy was associated with raised cortisol concentrations at 30 years of age (109).

Other work is in progress. For example, a follow up study of individuals exposed to glucocorticoids antenatally during treatment for preterm labour is underway in New Zealand. Examination of HPAA function in this cohort should provide important data to assess the relevance to human health of the numerous animal studies employing this model. Recruitment has recently finished for a large prospective study based in Southampton. Here, detailed information (diet, lifestyle, social circumstances, anthropometry, basic biochemistry etc) has been collected from women before conception and reassessed at regular intervals throughout pregnancy. Fetal data have also been recorded. These offspring will form a fascinating cohort in which to study programming phenomena, including HPAA activity.

The suggestion that HPAA activity is reduced, albeit in only one test, in low birthweight individuals from the new Hertfordshire cohort does not fit with the hypothesis that reduced fetal growth and adult cardiovascular disease may be linked by lifelong

overexposure to glucocorticoids. However, none of the markers of central HPAA function in this study correlated with the components of the metabolic syndrome, despite associations between fasting morning cortisol and cardiovascular risk factors similar to those seen in previous studies. These observations suggest that abnormalities at the level of the hypothalamus and pituitary do not underlie these relationships and further work is needed to determine the cause of raised circulating cortisol concentrations in individuals with the metabolic syndrome.

It is important to recognise that the three tests HPAA activity in Chapter 2 are not generally used in this type of population-based research. Normative data for similarly aged men are hard to find in the literature and where they exist they are often based on small numbers. The data collected in this study could therefore provide a valuable resource. Conditions most likely to influence HPAA activity were excluded (diabetes, depression and recent exposure to glucocorticoids), and it is likely that the range of morbidity within this sample was representative of other 'healthy' men in their sixties.

The second study (Chapter 3) was set up to examine the hypothesis that antenatal events resulting in small size at birth might program HPAA responses to stress. There is extensive animal data to support this idea (224, 225) and a more detailed investigation of cortisol secretion in a sample of men from the first Hertfordshire study suggested that this might also be the case in humans. This study found that there were no birthweight-related differences in diurnal cortisol profile over a 24 hour period, despite the strong inverse relationships that had been reported with 0900h cortisol (115). One interpretation of this apparently contradictory result is that a fasting morning cortisol taken in a novel clinic setting may reflect the reactivity of the axis rather than basal activity.

The Adelaide cohort was chosen for two reasons; as young adults they were free from potentially confounding morbidity and inverse associations between birthweight and cortisol had previously been reported in this population (111). The study did not demonstrate any convincing influence of size at birth on salivary cortisol responses to stress. The methodological problems encountered are discussed at length in Chapter 3. It is possible that a stronger psychological stimulus and a more detailed assessment of the response (blood sampling, improved subjective measures), having accurately established basal HPAA activity within the sample, may have provided better data with which to confirm or refute a role for programming of HPAA stress responsiveness in the fetal origins of adult disease with confidence. An important study, which hopes to address some of these issues, is ongoing in Southampton, using the Trier Social Stress Test in eight year old children. Detailed pregnancy data is available in this cohort, so the analysis will not have to rely solely on size at birth as the indicator of fetal exposures.

The assessment of haemodynamic reactivity detailed in Chapter 3 provided some novel and interesting data; blood pressure responses to psychological stress were strongly influenced by size at birth in women. This fits with animal data and may be an important clue to the mechanisms underlying the fetal programming of hypertension. Clearly, the findings need to be confirmed in other studies and future work should seek to determine which systems are upregulated in growth-restricted women. The autonomic nervous system is the most likely candidate. Heart rate data was collected continuously in the current study and work is underway to explore the signal further by spectral analysis. This should enable an assessment of heart rate variability and thus the relative activity of sympathetic and parasympathetic nervous systems over the course of the study.

Gender differences are frequently reported in the programming literature and thus the absence of a relationship between size at birth and haemodynamic stress reactivity in men should not detract from the significant result in women. They are difficult to explain and are an important question that needs to be addressed in future research. The answer is likely to lie in the interaction of sex steroids with physiological systems both during development and throughout life; a complex issue that may be difficult to unpick in human studies. A recent study in guinea pigs has provided a possible explanation for the sex differences often described in the HPAA programming literature. Distinct patterns of brain corticosteroid receptor (MR and GR) expression were found in the two sexes during fetal development, suggesting that males and females may be vulnerable to glucocorticoid exposure at different times (226).

The main aim of this thesis was to examine central regulation of the HPAA as the possible cause of hypercortisolaemia in low birthweight individuals. One important aspect was not examined in detail - feedback control. In animal models where the HPAA is upregulated by prenatal manipulations, hippocampal corticosteroid receptor expression is often reduced, suggesting that impaired negative feed back may responsible for the HPAA overactivity (72, 94, 95).

Feedback sensitivity is traditionally examined in humans using the synthetic steroid, dexamethasone. In Chapter 2, the 1.5mg overnight dexamethasone suppression test did not differentiate between the birthweight groups. This was not surprising given that a previous study found no association between birthweight and the results of a 0.25mg DST; a dose chosen to discriminate between individuals without overt HPAA pathology (117). On the

basis of these results, it is reasonable to assume that feedback at the level of the pituitary is not affected by prenatal events but, as dexamethasone does not freely cross the blood brain barrier (153), little can be inferred about hippocampal feedback.

It had been hoped that this question could be addressed as part of the Adelaide study, using a prednisolone suppression test. This test has been worked up by our group and others as a more physiological method of assessing feedback sensitivity (ie including the hippocampus). At a dose that suppresses the axis by 50% (4-5mg), it appears to be reproducible and to offer different information to the low dose DST (227, 228). Unfortunately, despite gaining ethical approval, the regulatory authorities in Australia would not permit the use of this test as it fell outside the license for prednisolone. Ethical approval has since been obtained to perform this study in the new Hertfordshire cohort.

Circulating cortisol concentrations are determined by the relative rates of secretion and metabolism. Thus, it is possible that altered cortisol metabolism underlies the association between impaired fetal growth and hypercortisolaemia. There was no evidence that urinary cortisol metabolite ratios varied according to birthweight in one study (117), but a direct approach may yield more useful results. A preliminary study is underway in Southampton to determine whether the disappearance of a bolus of deuterated cortisol is a test that may be useful in cohort studies in the future.

Gene-environment interactions are an area of increasing interest in this field of research. For example, Eriksson and colleagues have reported that the inverse relationship between birthweight and insulin resistance was only seen in those individuals carrying an allele of the PPARγ gene that is known to be associated with an increased risk of type 2 diabetes

(229). One potential mechanism whereby environmental exposures may influence gene expression is via differential methylation (230, 231). Genetic material has been collected from members of the new Hertfordshire cohort which may enable further interpretation of the data described in Chapter 2 in the future.

Post natal growth and environment also influence prenatal exposures. In the context of the metabolic syndrome, the dramatic rise in the prevalence of obesity in recent years is likely to be particularly important. Maternal protein restriction in rats has much graver consequences for the offspring if they subsequently become obese (45). Similarly, evidence of an interaction between HPAA activity and obesity in predicting cardiovascular risk factors has been found in several studies, including the present work. Longitudinal studies will provide data to examine these factors in more detail than has been possible in the current work.

The driving force behind all medical research is the desire to reduce the burden of disease in society. Thus, it is vitally important that the 'fetal origins' hypothesis moves beyond epidemiology, as interventions to improve public health can only be instituted when mechanisms have been identified. Animal models have an important role to play in this as studies at a molecular and cellular level are easier to perform, but without human data to support the findings in other species interventions would be unethical. This underlines the importance of the current work.

In summary, the work presented in this thesis has added complexity to the role of the HPAA in the fetal origins of adult disease, and confirms that this is likely to remain an exciting area of research in years to come.

APPENDIX A

HERTFORDSHIRE 31-39 HEALTH QUESTIONNAIRE

Name: _____

Address: _____

_____

_____

Telephone: _____

GP _____

Interviewer _____

Date of Interview:

d	d	m	m	y	y

Date last modified 09/11/00

SECTION 1   GENERAL

Q1   What is your date of birth?

d   d   m   m   y   y

Q2   Where were you born?   _____

Q3   Are you   1.   Single?

2.   Married?

3.   Divorced or separated?

4.   Widowed?

5.   Cohabiting?

Q4a   What is your current or most recent full-time
job?   *(Probe if necessary)*

_____

b   What industry was that in?

_____

If an ever married woman, continue, otherwise go to Q7

Q5   What was your maiden name?

_____

Q6a   What is/was your husband's current or most
recent full-time job?   *(Probe if necessary)*

_____

b   What industry was that in?

_____

Q7   Please count the number of rooms your household has for its own use.
Do not count: small kitchens (under 2 metres wide), bathrooms or toilets
Do count: Living rooms, kitchens (at least 2 metres wide) bedrooms and all
other rooms

The total number of rooms is:

Q8   Is your accommodation owned/mortgaged or rented by your household?

Owned/Mortgaged

Rented

Other

Q9   How old were you when you finished continuous full-time education?

years old

Q10   How many cars and vans would you normally have available for use by you or other members of your household?

None

One

Two

Three or more

SECTION 2   FAMILY HISTORY

Q11   Where was your mother born?

_____

Q12   What was your mother's year of birth?

_____

Q13   Where was your father born?

_____

Q14   What was your father's year of birth?

_____

Q15 How many babies did your mother have? *(including stillbirths and babies that died)*

Order	Name	Date of birth	Sex	Year of death	Cause of death	Live in Herts Y/N

SECTION 3 - CHILDHOOD

Q16 What was your father's job when you were born? (if unemployed, last full-time job)

_____

Q17 Did your father smoke regularly during your childhood?

0. No 1. Yes ☐

Q18 Did your mother smoke regularly during your childhood?

0. No 1. Yes ☐

Q19 Did your family have a cat when you were a child?

0. No 1. Yes ☐

Q20 Did your family keep a dog when you were a child?

0. No 1. Yes ☐

Q21 Did you share a bedroom with your brothers/sisters before you started school?

0. No 1. Yes ☐

Q22   Do you know how much you weighed when you were born?

0. No 1. Yes

If yes, how much did you weigh?

..............lbs   ....ozs

Q23   Were you born early, on time, or late?

SECTION 4   PHYSICAL ACTIVITY

Q24   Do you have any problems walking?

0.   No limiting abnormality
1.   Abnormal gait/walking problems/no aid
2.   Uses walking aid
3.   Requires help from another person
4.   Unable to walk

Q25a   **Walking out of doors:** record all walking yesterday lasting longer than 5 minutes

i   Before 9.00 a.m.   mins

ii   Between 9.00 a.m. and 12.00 p.m.   mins

Iii Between 12.00 p.m. and 2.00 p.m.   mins

iv   Between 2.00 p.m. and 6.00 p.m.   mins

v   Between 6.00 p.m. and 7.00 p.m.   mins

vi   After 7.00 p.m.   mins

vii Total   mins

b   Was this day unusual?

0. No   1. Yes

If yes, did you walk less or more than usual?

1. Less   2. More

Q26   Which of the following best describes your walking speed?

    0.   Unable to walk
    1.   Very slow
    2.   Stroll at an easy pace
    3.   Normal speed
    4.   Fairly brisk
    5.   Fast

Q27   Which of the following activities do you do at least once a month on average or at least 12 times per year?

Bowls	0. No	1. Yes
Cycling	0. No	1. Yes
Swimming	0. No	1. Yes
Golf	0. No	1. Yes
Fishing	0. No	1. Yes
Dancing	0. No	1. Yes
Other physically active sports	0. No	1. Yes

or hobbies except gardening
(please specify)

Q28   How much time do you spend gardening in a typical week?

    0.   Less than 1 hour per week
    1.   1-4 hours per week
    2.   5-8 hours per week
    3.   More than 8 hours per week

Q29   How much time do you spend doing housework in a typical week?

    0.   Less than 1 hour per week
    1.   1-4 hours per week
    2.   5-8 hours per week
    3.   More than 8 hours per week

Q30 Do you climb stairs?

    0.   Never
    1.   Occasionally
    2.   Once/several times per week
    3.   Daily
    4.   Several times per day

Q31 Do you carry loads (equivalent to a full shopping bag or 10 lbs)?

    0.   Never
    1.   Occasionally
    2.   Once/several times per week
    3.   Daily
    4.   Several times per day

Q31a Have you had any falls in the last year?

                                          0. No   1. Yes

    b *If yes,* how many?

SECTION 5 - SOCIAL

Q32a Have you ever smoked regularly?
(i.e. at least once a day for a year or more)

                                          0. No   1. Yes

    If yes, continue
    If no,   Go to Q34

  B How old were you when you first smoked regularly?

  C If you added up all the years that you smoked, how many would it make in total?

  D What was the average amount you smoked over this time?

    Cigarettes/day

    Roll-ups (ozs)/week

    Cigars/week

    Pipe tobacco (ozs)/week

e   Do you still smoke regularly?

0. No   1. Yes   ☐

>   *If yes,*   Go to Q33
>   *If no,*   continue

f   How old were you when you last smoked regularly?

☐☐

Q33   How much do you smoke now?

Cigarettes/day

☐☐☐

Roll-ups tobacco/week (oz)

☐☐

Cigars/week

☐☐

Pipe tobacco/week (oz)

☐☐

If appropriate, between what ages did you cut down?

☐☐ to ☐☐

Q34a   Apart from your own smoking are you regularly exposed to tobacco smoke at home?

0. No   1. Yes   ☐

If yes,

b   Not counting yourself, how many people in your household smoke regularly?

☐☐

Q35a   Do you ever drink alcohol?

0. No   1. Yes   ☐

If no, go to 36a

How often do you currently drink shandy/low alcohol
beer/lager/cider? (don't include alcohol free lager etc.)

0.   Never

1.   Once every 2-3 months

2.   Once a month

3.   Once a fortnight

4.   1-2 times per week

5.   3-6 times per week

6.   Once a day

7.   More than once a day

When you drink these, how many pints would you normally have? (if range
given code mid-point; 1 average can = 0.8 pints, 1 small can = 0.5 pints)

35b   How often do you currently drink beer/stout/lager/cider? (don't
include alcohol free lager etc.)

0.   Never

1.   Once every 2-3 months

2.   Once a month

3.   Once a fortnight

4.   1-2 times per week

5.   3-6 times per week

6.   Once a day

7.   More than once a day

When you drink these, how many pints would you normally have? (if range
given code mid-point; 1 average can = 0.8 pints, 1 small can = 0.5 pints)

35c   How often do you currently drink low alcohol wine?

0.   Never

1.   Once every 2-3 months

2.   Once a month

3.   Once a fortnight

4.   1-2 times per week

5.   3-6 times per week

6.   Once a day

7.   More than once a day

When you drink these, how many glasses would you normally have? (if range given code mid-point)

35d   How often do you currently drink Wine/Sherry/Port /Martini /Cinzano?

0.   Never

1.   Once every 2-3 months

2.   Once a month

3.   Once a fortnight

4.   1-2 times per week

5.   3-6 times per week

6.   Once a day

7.   More than once a day

When you drink these, how many glasses would you normally have? (if range given code mid-point)

35e How often do you currently drink spirits/liqueurs?

0. Never

1. Once every 2-3 months

2. Once a month

3. Once a fortnight

4. 1-2 times per week

5. 3-6 times per week

6. Once a day

7. More than once a day

When you drink these, how many measures would you normally have? (if range given code mid-point)

SECTION 6 – CHEST PAIN

Q36a Do you get pain or discomfort in your chest

1. Yes	*go to c*
0. No	*go to b*

b Do you get any pressure or heaviness in your chest?

1. Yes	*go to c*
0. No	*go to l*

c Do you get it when you walk uphill or hurry?

0. No
1. Yes
2. Never hurry or walk uphill

d Do you get it when you walk at an ordinary pace on the level?

0. No   1. Yes

If No to c and d, *go to h*

e What do you do if you get it while you are walking?

1. Stop or slow down
2. Carry on

(Record stop or slow down if the subject carried on after taking nitro-glycerine)

153

f If you stand still or slow down what
happens to it?

1. Relief

0. No relief

g How long does it take to get relief?

1. 10 minutes or less

2. More than 10 minutes

h Will you show me where it was? Note the number(s)
of the site(s) from the chest diagram

i Do you feel it anywhere else?

0. No   1. Yes

If yes, please specify

j Did you see a doctor because of this pain/discomfort

0. No   1. Yes

If yes, what did he/she say that it was?

k How many years ago did this pain or discomfort start?

l Have you ever had severe pain across the front of your chest
lasting for half an hour or more?

0. No   1. Yes

If yes, go to m, *if no* go to o

m Did you see a doctor because of this pain?

0. No   1. Yes

If yes, what did he/she say that it was?

n   How many of these attacks/episodes have you had?

1.   Date 1 (year) _____   Duration of pain _____

2.   Date 2 (year) _____   Duration of pain _____

3.   Date 3 (year) _____   Duration of pain _____

If subject feels unsure enter 9 here

o   Have you ever had an operation to clear the arteries in your heart
(coronary artery bypass graft or angioplasty)?

0. No   1. Yes

If yes, go to p, *if no* go to q

p   In what year did it occur for the first time?

q   Have either of your parents or any of your brothers or sisters suffered from a heart
attack?

0. No   1. Yes

If yes, please give details

Relative	Age of first attack

Q37a Do you get pain or discomfort in your legs when you walk?

0. No   1. Yes   ☐

If no, go straight to Q39

b Does this pain ever begin when you are standing still or sitting?

0. No   1. Yes   ☐

c Do you get it when you walk uphill or hurry?

0. No   1. Yes   ☐

d Do you get it when you walk at an ordinary pace on the level?

0. No   1. Yes   ☐

e What do you do if you get it when you are walking?

1. Stop ☐   2. Slow down ☐   3. Continue at   ☐
                                                 same pace

f Does the pain ever disappear while you are still walking?

0. No   1. Yes   ☐

g What happens to it if you stop or slow down?   ☐

1. Usually continues for more than 10 minutes

2. Usually disappears in 10 minutes

h Where do you get this pain or discomfort? (*show card and tick box*)

1. Calf ☐   2. Thighs ☐   3. Buttock ☐

4. Groin ☐   5. Knee ☐   6. Ankle ☐

Q38 Have you ever had surgery to your aorta or to the arteries in your legs?

0. No   1. Yes   9. Don't know   ☐

SECTION 7 – RESPIRATORY

Cough

Q39a   Do you **usually** cough first thing in the
morning in winter?   0. No   1. Yes

b   Do you **usually** cough during the day -
or at night in the winter?   0. No   1. Yes

If yes, go to c, *if no,* go to d

c   Do you cough like this on most days for
as much as 3 months of each year?   0. No   1. Yes

Phlegm

d   Do you **usually** bring up any phlegm
from your chest first thing in the morning   0. No   1. Yes
in winter?

e   Do you **usually** bring up any phlegm
from your chest during the day or at   0. No   1. Yes
night in the winter

If yes, go to f, *if no,* go to Q40a

f   Do you bring up phlegm like this on most
days for as much as 3 months each year?   0. No   1. Yes

Q40a   Have you had wheezing or whistling in your
chest at any time during the last year   0. No   1. Yes

If yes, continue, *if no,* go to Q40b
i)   Have you had this wheezing when you   0. No   1. Yes
did not have a cold?

ii)   Have you been at all breathless when   0. No   1. Yes
the wheezing noise was present?

b   Have you woken with a feeling of chest
tightness first thing in the morning at any   0. No   1. Yes
time in the last year?

Q41   Have you been woken by an attack of shortness
of breath at any time during the last year   0. No   1. Yes

Q42a   Are you often troubled by shortness of breath

157

| | when hurrying on level ground or walking up a slight hill? | 0. No | 1. Yes | ☐ |

If yes, continue, *if no,* go to Q43

| b | Do you often get short of breath walking with other people of your own age on level ground? | 0. No | 1. Yes | ☐ |

If yes, continue, *if no,* go to Q43

| c | Do you often have to stop for breath when walking at your own pace on level ground? | 0. No | 1. Yes | ☐ |

If yes, continue, *if no,* go to Q43

| d | Do you often have to stop for breath after walking about 100 yards (or after a few minutes) on the level? | 0. No | 1. Yes | ☐ |

If yes, continue, *if no,* go to Q43

| e | Do you get breathless on washing or dressing? | 0. No | 1. Yes | ☐ |

| Q43 | Have you had to see your doctor in the last year for your chest | 0. No | 1. Yes | ☐ |

| | Have you been admitted to hospital for your chest in the last year? | 0. No | 1. Yes | ☐ |

Q44 What kind of cooker do you MOSTLY use for cooking? *(Choose one method only)*

1. Gas 2. Electricity   3. Other (specify below)   ☐

SECTION 8 – IMMUNITY

Q45   Did you have eczema as a child?

                                              0. No   1. Yes   ☐

Q46   Have you ever had hay fever, rhinitis or other
           nasal allergies?   0. No   1. Yes   ☐

Q47   Have you ever had glandular fever?

                                              0. No   1. Yes   ☐

           If yes, at what age?   ☐☐

Q48   Have you ever had your appendix out?

                                              0. No   1. Yes   ☐

           If yes, at what age?   ☐☐

Q49   Have you ever had shingles?

                                              0. No   1. Yes   ☐

           If yes, at what age?   ☐☐

Q50   Have you ever had hepatitis A vaccine e.g. for
           travel purposes?   0. No   1. Yes   ☐

Q51   Have either of your parents, or any of your brothers or
           sisters ever had asthma, hayfever or childhood eczema?

                                              0. No   1. Yes   ☐

           If yes, please give details

Relative	Illness

SECTION 9 – BONE

Q52 Have you broken any bones since the age of 45?

If yes, please give details   0. No   1. Yes

Bone	Age when fracture occurred	How did fracture occur?

Q53 Have either of your parents or any of your brothers or sisters fractured a bone when they were more than 45 years old?

If yes, please give details   0. No   1. Yes

Which relative?	Bone	Age when fracture occurred	How did fracture occur?

Q54 Have you ever had back pain in the area shown on the card, which lasted for more than a day? *(do not include pain occurring only during pregnancy, during menstrual periods, or during the course of a feverish illness such as flu)*

     0. No   1. Yes

If yes, please answer questions below
If no, go to Q57

Q55 Has the pain ever spread to your legs?

     0. No   1. Yes

If yes, please tell me the furthest point down your leg that the pain reached

Buttock

Thigh

Knee

Calf

Ankle

Q56   When did you last have the pain?

Last week

Last month

Last year

More than a year ago

Occupational History

Q57   Record all jobs/occupations of greater than 1 years duration since the person left full-time education.

Job Title	Age Started	Age stopped	Part time/ Full time	Activity		
				Standing	Lifting	Sweating

Record in activity column if the job involved:
1.   Standing/walking for 4+ hours per day
2.   Lifting 25kg +
3.   Physical work enough to make the subject sweat

SECTION 10   OBSTETRIC

MEN ONLY

Q58   How many children have you fathered?

*WOMEN ONLY. **For men go to** SECTION 11*

Q59   How many times have you been pregnant?

Details:

Pregnancy Number	Liveborn (L) Stillborn (S) Miscarriage(M)	If liveborn:		Currently living in Herts	
		Male (M) Female (F)	Birthweight	Name	D.O.B.
1					
2					
3					
4					
5					
6					
7					
8					

Q60a At what age did your periods start?

b At what age did your periods stop?

c Have you had a hysterectomy (removal of the womb)?

0. No   1. Yes

d *If yes* how old were you?

e Did the hysterectomy include removal of the ovaries?

0. No   1. Yes   2. Don't know

Q61a Have you ever taken an oral contraceptive pill?

0. No   1. Yes

b *If yes,* at what age did you start?

c   How long in total did you take it for (months)?

Q62a   Have you ever taken hormone replacement therapy?

0. No   1. Yes

b   *If yes,* at what age did you start?

c   How long in total did you take it for (months)?

Q63   have you ever taken tamoxifen (eg for a breast lump)?

0. No   1. Yes   9. Don't know

SECTION 11 – MEDICAL

Q64   Have you ever been told by a doctor or other health professional that you have ever had any of the following:-

a   High blood pressure (out of pregnancy only)

0. No   1. Yes   9. Don't know

b   Stroke/Transient ischaemic attack

0. No   1. Yes   9. Don't know

c   Diabetes (out of pregnancy)

0. No   1. Yes   9. Don't know

If yes, how long have you been diabetic?

Years

Are you controlled by:

Diet alone

Tablets

Insulin injections

163

d Have you ever had a head injury severe enough to cause unconsciousness or to require admission to hospital?

0. No   1. Yes   9. Don't know

Q65 Have either of your parents or any of your brothers or sisters had high blood pressure or diabetes?

0. No   1. Yes   9. Don't know

If yes, please give details

Which relative?	Illness	Age when illness occurred	Form of treatment

SECTION 12 - MEDICATION

Q66 What regular medicines/tablets/eye drops/inhalers etc. do you use?
PLEASE USE BLOCK CAPITALS
Please include regular pain killers such as paracetamol

1 _____

2 _____

3 _____

4 _____

5 _____

6 _____

7 _____

8 _____

9 _____

10 _____

11 _____

12 _____

SECTION 13   HEALTH AND DAILY ACTIVITIES

Q67   In general how would you say your health is:

1.	Excellent	2.	Very good
3.	Good	4.	Fair
5.	Poor		

Q68   Compared to one year ago, how would you rate your health in general now?
Please indicate only one

1.	Much better than one year ago	2.	Somewhat worse than one year ago
3.	Somewhat better than one year ago	4.	Much worse than one year ago
5.	About the same as one year ago		

Q69   The following items are about activities you might do during a typical day
Does **your health now limit you** in these activities? If so, please indicate
how much?

	Yes limited a lot	Yes limited a little	No, not limited at all
a) **Vigorous activities**, such as running, lifting heavy objects, Participating in strenuous sports	☐	☐	☐
b) Moderate activities, such as moving a table, pushing a vacuum cleaner, Bowling or playing golf	☐	☐	☐
c) Lifting or carrying groceries	☐	☐	☐
d) Climbing **several** flight of stairs	☐	☐	☐
e) Climbing **one** flights of stairs	☐	☐	☐
f) Bending, kneeling or stooping	☐	☐	☐
g) Walking **more than one mile**	☐	☐	☐
h) Walking **half a mile**	☐	☐	☐
i) Walking **one hundred yards**	☐	☐	☐
j) Bathing or dressing yourself	☐	☐	☐

Q70 During the **past 4 weeks,** have you had any of the following problems with your work or other regular daily activities **as a result of your physical health?** *Please indicate one answer for each question*

a) Cut down the **amount of time** you spent on work or other activities

0. No 1. Yes

b) **Accomplished less** than you would like

0. No 1. Yes

c) Were limited in the **kind** of work or other activities

0. No 1. Yes

d) Had **difficulty** performing the work or other activities (for example, it took extra effort)

0. No 1. Yes

Q71 During the **past 4 weeks,** have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems?** *Please indicate one answer for each question*

a) Cut down the **amount of time** you spent on work or other activities

0. No 1. Yes

b) **Accomplished less** than you would like

0. No 1. Yes

c) Didn't do work or other activities as **carefully** as usual.

0. No 1. Yes

Q72 During the **past 4 weeks,** to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours or groups? *Please indicate one only*

1. Not at all   2. Slightly

3. Moderately   4. Quite a bit

5. Extremely

Q73 During the **past 4 weeks,** how much bodily pain have you had? *Please indicate one only*

1. None   2. Very mild

3. Mild   4. Moderate

5. Severe   6. Very severe

Q74 During the **past 4 weeks,** how much did **pain** interfere with your normal work (including both work outside the home and housework)? *Please indicate one only*

1.	Not at all	2.	A little bit
3.	Moderately	4.	Quite a bit
5.	Extremely		

Q75 During the **past 4 weeks,** how much of the time?
Please indicate one answer for each question

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
a) Did you feel full of life?						
b) Have you been a very Nervous person?						
c) Have you felt so down in the dumps that nothing could cheer						
d) Have you felt calm and peaceful?						
e) Did you have a lot of energy?						
f) Have you felt downhearted and low?						
g) Did you feel worn out?						
h) Have you been a happy person?						
i) Did you feel tired						

Q76 During the **past 4 weeks,** how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting friends, relatives, etc)? *Please indicate one only*

1.	All of the time	2.	Most of the time
3.	Some of the time	4.	A little of the time
5.	None of the time		

Q77 Please choose the answer that best describes how **TRUE or FALSE** each of the following statements is for you. *Please indicate one answer for each question.*

	Definitely True	Mostly True	Don't Know	Mostly False	Definitely false
a) I seem to get sick a little easier Than other people	☐	☐	☐	☐	☐
b) I am as healthy as anybody I know	☐	☐	☐	☐	☐
c) I expect my health to get worse	☐	☐	☐	☐	☐
d) My health is excellent	☐	☐	☐	☐	☐

SECTION 14

"The following questions will help you to let us know how you are. Please give the response which comes closest to how you have felt in the last few days. Don't take too long over your replies, your immediate reaction will probably be more accurate than a long thought out response"

Q78 I feel tense or 'wound up':

1.	Most of the time	2.	A lot of the time	☐
3.	From time to time, occasionally	4.	Not at all	

Q79 I feel as if I am slowed down:

1.	Nearly all the time	2.	Very often	☐
3.	Sometimes	4.	Not at all	

Q80 I still enjoy the things I used to enjoy:

1.	Definitely as much	2.	Not quite so much	☐
3.	Only a little	4.	Hardly at all	

Q81 I get a sort of frightened feeling like butterflies in the stomach:

1.	Not at all	2.	Occasionally	☐
3.	Quite often	4.	Very often	

Q82 I get a sort of frightened feeling as if something awful is about to happen::

1.	Very definitely and quite badly	2.	Yes, but not too badly	☐
3.	A little bit but it doesn't worry me	4.	Not at all	

Q83   I have lost interest in my appearance:

    1. Definitely   2. I don't take so much care as I should

    3. I may not take quite as much care   4. I take just as much care as ever

Q84   I can laugh and see the funny side of things:

    1.   As much as I always could   2.   Not quite so much now

    3.   Definitely not so much now   4.   Not at all

Q85   I feel restless as if I have to be on the move:

    1.   Very much indeed   2.   Quite a lot

    3.   Not very much   4.   Not at all

Q86   Worrying thoughts go through my mind:

    1.   A great deal of time   2.   A lot of the time

    3.   From time to time but not too   4.   Only occasionally
          often

Q87   I look forward with enjoyment to things:

    1.   As much as I ever did   2.   Rather less than I used to

    3.   Definitely less than I used to   4.   Hardly at all

Q88   I feel cheerful:

    1.   Not at all   2.   Not often

    3.   Sometimes   4.   Most of the time

Q89   I get sudden feelings of panic:

    1.   Very often indeed   2.   Quite often

    3.   Not very often   4.   Not at all

Q90   I can sit at ease and feel relaxed:

    1.   Definitely   2.   Usually

    3.   Not often   4.   Not at all

Q91   I can enjoy a good book or radio or TV programme:

    1.   Often   2.   Sometimes

    3.   Not often   4.   Very seldom

Thank you

APPENDIX B

STRESS AND CORTISOL STUDY QUESTIONNAIRE

SUBJECT ID: **DATE:**

In order to interpret the biological data we are collecting from you today we need to ask you for some details of your health and lifestyle. There are also some questions that relate to your attitudes and opinions and the way you feel about yourself. You may feel that some of the questions do not apply to you, but please answer each question with the response that most closely fits the way you feel.

The answers you provide in this questionnaire will be kept **strictly confidential**. The information will go into the analysis of the study, but it will not be possible to identify your individual responses from any reports or publications. Under no circumstances will any information be made available to anyone else.

Many of the questions can be answered by ticking or circling the appropriate answer. Please use a blue pen.

Please be sure to read the instructions to each section carefully.

Thank you very much for your participation

PERSONAL DETAILS   ID:

1. **Date of birth**   □□/□□/□□□□

2. **Sex**

    Male   □₁
    Female   □₂

3. **Postcode of your usual place of residence**   □□□□

4. **What level did you reach at school**

    Completed primary school   □ 0
    Some high school (eg to year 10) □ 1
    Completed high school   □ 2

5. **Have you completed any tertiary education**

    No   □ 0
    Qualification from a TAFE college or similar □ 1
    University degree   □ 2

6. **Which of the following describes your current employment status?**
    Please tick more than one box where applicable.

    Working full-time (permanent or contract)   □ 0
    Working part-time (permanent or contract)   □ 1
    Casual full-time   □ 2
    Casual part-time   □ 3
    Unemployed   □ 4
    Home duties   □ 5
    Full-time student   □ 6
    Part-time student   □ 7
    Permanently unable to work / ill   □ 8
    Other (please specify)   □ 9

    ....

7. **If you have a full or part-time job of any kind, please give a description of your main job**
    Please give the full title and outline the main tasks or duties that you perform.

    Title: ....

    Main tasks: ....

8. **How many hours a week do you work?**   □□

9. **Are you:**

    Single (never married)   □ 0
    Married / De facto (living with someone) □ 1
    Separated   □ 2
    Divorced   □ 3
    Widowed   □ 4

171

SMOKING / ALCOHOL HISTORY

10. How often do you CURRENTLY smoke cigarettes or other tobacco products?

Not at all $\qquad$ ☐0
Less often than weekly $\qquad$ ☐1
At least weekly (not daily) $\qquad$ ☐2
Daily $\qquad$ ☐3

If **not at all**, in your lifetime have you smoked over 100 cigarettes or similar
No *(Go to Q14)* ☐0
Yes *(Go to Q 12)* ☐1

11. How often, if at all, do you CURRENTLY smoke:

		Not at all	Less than weekly	At least weekly	Daily	Number Day	Week
a)	Manufactured cigarettes	1	2	3	4		
b)	Roll up cigarettes	1	2	3	4		
c)	Cigars	1	2	3	4		
d)	Pipe tobacco	1	2	3	4		

12. Have you EVER smoked tobacco daily?

No, I have never smoked daily *(Go to Q 14)* ☐0
Yes, I still smoke daily *(Only tick if you have answered daily to a previous question)* ☐1
Yes, I used to smoke daily ☐2
When did you finally stop smoking tobacco daily

....

13. At what age did you first start smoking daily?

....

14. How often do you USUALLY drink alcohol?

I don't drink alcohol *(Go to Q 17)* ☐0
Less than once a week ☐1
On 1 day a week ☐2
On 2 or 3 days a week ☐3
On 4 to 6 days a week ☐4
Every day ☐5

15. On a day when you drink alcohol, how many STANDARD drinks do you usually have?

1 or 2 drinks ☐1
3 or 4 drinks ☐2
5 to 6 drinks ☐3
7 to 8 drinks ☐4
9 to 12 drinks ☐5
13 or more drinks ☐6

A standard drink is:
1 schooner full-strength beer
1 pint light beer
1 small glass wine
1 glass port
1 nip spirits

16. What do you usually drink?

....

EXERCISE HISTORY

In the next three questions we want to find out about the EXERCISE you have had during the PAST 2 WEEKS:
➢   for recreation, sport or health-fitness purposes
➢   as part of your tasks at work and around the house

Please distinguish between vigorous exercise which made you breathe harder or puff and pant, and less vigorous exercise.

17. **In the PAST 2 WEEKS did you engage in VIGOROUS EXERCISE – exercise that made you breathe harder or puff and pant?**
    (eg. Vigorous sports such as football, netball, tennis, squash, athletics; jogging or running; keep-fit exercises; vigorous swimming etc)

    No   ☐₀
    Yes   ☐₁
    If yes:
    How many sessions of vigorous exercise did you do over the 2 week period   ☐☐
    How many hours did you spend exercising vigorously during the past 2 weeks   ☐☐

18. **In the PAST 2 WEEKS did you engage in LESS VIGOROUS EXERCISE for recreation, sport or health-fitness purposes which did not make you breathe harder or puff and pant?**
    (eg Walking, gentle swimming, yoga)

    No   ☐₀
    Yes   ☐₁
    If yes:
    How many sessions of less vigorous exercise did you do over the 2 week period   ☐☐

19. **In the PAST 2 WEEKS did you engage in VIGOROUS ACTIVITY, apart from exercise, which made you breathe harder or puff and pant?**
    (eg. Carrying loads, heavy gardening, chopping wood, labouring – at home, during employment or anywhere else)

    No   ☐₀
    Yes   ☐₁
    If yes:
    How many sessions of this type of exercise did you have over the 2 week period   ☐☐
    How many hours did you spend doing vigorous activities during the past 2 weeks   ☐☐

MEDICAL HISTORY

20. In general would you say your health is:

Excellent	☐ 1
Very good	☐ 2
Good	☐ 3
Fair	☐ 4
Poor	☐ 5

21. Do you have any longstanding illnesses, diseases or medical conditions for which you have sought treatment in the last 12 months?

No	☐ 0
Yes	☐ 1

If yes, please detail

....

....

....

22. Have you ever suffered from any of the following conditions?

Asthma / eczema / hayfever	☐ 1
Epilpesy	☐ 2
Diabetes	☐ 3
Thyroid disease	☐ 4
Pituitary disease	☐ 5
Addison's disease	☐ 6
Heart disease	☐ 7
Depression / anxiety	☐ 8
Eating disorder	☐ 9

If yes to any of the above, please give details:

....

....

....

23. Have you taken any form of medication (prescribed, over the counter, herbal) in the last 3 months?

No	☐ 0
Yes	☐ 1

If yes, please list:

....

....

....

24. Have you used medication containing steroids, for example asthma inhalers, eczema creams, or tablets for severe inflammation in the last five years?

   No   ☐0
   Yes   ☐1
   If yes, please detail what type and when you last took any:

   ....

   ....

25. Are there any major distressing situations in your life at the moment?

   No   ☐0
   Yes   ☐1
   If yes, please give details

   ....

   ....

Women Only:

26. What was the date of the first day of your last period?   ☐☐/☐☐/☐☐☐☐

27. Are you on 'the pill', or any other form of hormonal contraception (eg injections)?

   No   ☐0
   Yes   ☐1

28. Is there any possibility that you may be pregnant?

   No   ☐0
   Yes   ☐1

INFORMATION FROM TODAY

29. Did you sleep well last night?

No ☐ 0
Yes ☐ 1

30. What time did you wake? ☐☐.☐☐

31. Did you go to work this morning?

No ☐ 0
Yes ☐ 1

32. How did you reach the hospital / were there any problems getting here?

....

....

33. What time did you last eat? ☐☐.☐☐

34. What did you eat?

....

....

35. What time did you last have a drink containing caffeine?

Date .... Time: ☐☐.☐☐

36. If you smoke, what time did you last have a cigarette?

Date .... Time: ☐☐.☐☐

37. If you drink alcohol, when did you last drink any alcohol?

Date .... Time: ☐☐.☐☐

ATTITUDES AND FEELINGS

This section asks about your feelings during the <u>past week</u>.

Please circle the score for each statement that best describes how often you felt this way during the last week.

	Rarely or none of the time (less than 1 day)	Some or a little of the time (1-2 days)	Occasionally or a moderate amount of time (3-4 days)	Most or all of the time (5-7 days)
1. I was bothered by things that usually don't bother me	0	1	2	3
2. I did not feel like eating; my appetite was poor	0	1	2	3
3. I felt that I could not shake off the blues, even with help from my family and friends	0	1	2	3
4. I felt that I was just as good as other people	0	1	2	3
5. I had trouble keeping my mind on what I was doing	0	1	2	3
6. I felt depressed	0	1	2	3
7. I felt that everything I did was an effort	0	1	2	3
8. I felt hopeful about the future	0	1	2	3
9. I thought my life had been a failure	0	1	2	3
10. I felt fearful	0	1	2	3
11. My sleep was restless	0	1	2	3
12. I was happy	0	1	2	3
13. I talked less than usual	0	1	2	3
14. I felt lonely	0	1	2	3
15. People were unfriendly	0	1	2	3
16. I enjoyed life	0	1	2	3
17. I had crying spells	0	1	2	3
18. I felt sad	0	1	2	3
19. I felt that people dislike me	0	1	2	3
20. I could not "get going"	0	1	2	3

Thank you very much for completing this questionnaire.

APPENDIX C

STRESS AND CORTISOL STUDY – Rest Questionnaire

SUBJECT ID: **DATE:**

Please answer the following questions by circling the number that best describes the way you felt during the task.

1. How relaxed do you feel at the moment?

Not at all relaxed						**Very relaxed**
1	2	3	4	5	6	7

2. How anxious do you feel at the moment?

Not at all anxious						**Very anxious**
1	2	3	4	5	6	7

3. How stressed do you feel at the moment?

Not at all stressed						**Very stressed**
1	2	3	4	5	6	7

STRESS AND CORTISOL STUDY - Task impact questionnaire

SUBJECT ID: **DATE:**

Please answer the following questions by circling the number that best describes the way you felt during the task.

1. How difficult did you find the task?

**Not at all
difficult**   **Very
difficult**

    1   2   3   4   5   6   7

2. How involved in the task did you feel?

**Not at all
involved**   **Very
involved**

    1   2   3   4   5   6   7

3. How well do you think you performed the task?

**Not at all
well**   **Very
well**

    1   2   3   4   5   6   7

4. How stressed did you feel during the task?

**Not at all
stressed**   **Very
stressed**

    1   2   3   4   5   6   7

5. How much in control of the task did you feel?

**Not at all
in control**   **Very in
control**

    1   2   3   4   5   6   7

6. How relaxed did you feel during the task?

**Not at all
relaxed**   **Very
relaxed**

    1   2   3   4   5   6   7

REFERENCES

1. Murray, CJ and Lopez, AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. *Lancet* (1997) 349 1269-1276.

2. Murray, CJ and Lopez, AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet* (1997) 349 1498-1504.

3. Ebrahim, S and Smith, GD. Systematic review of randomised controlled trials of multiple risk factor interventions for preventing coronary heart disease. *Brit.Med.J.* (1997) 314 1666-1674.

4. Barker, DJP and Osmond, C. Infant mortality, childhood nutrition and ischaemic heart disease in England and Wales. *Lancet* (1986) 1 1077-1081.

5. Barker, DJP, Osmond, C, Winter, PD, Margetts, BM, and Simmonds, SJ. Weight in infancy and death from ischaemic heart disease. *Lancet* (1989) 2 577-580.

6. Osmond, C, Barker, DJP, Winter, PD, Fall, CHD, and Simmonds, SJ. Early growth and death from cardiovascular disease in women. *Brit.Med.J.* (1993) 307 1519-1524.

7. Martyn, CN, Barker, DJ, and Osmond, C. Mothers' pelvic size, fetal growth, and death from stroke and coronary heart disease in men in the UK. *Lancet* (1996) 348 1264-1268.

8. Leon, DA, Lithell, HO, Vagero, D, Koupilova, I, Mohsen, R, Berglund, L et al. Reduced fetal growth rate and increased risk of death from ischaemic heart disease: cohort study of 15 000 Swedish men and women born 1915-29. *Brit.Med.J.* (1998) 317 241-245.

9. Rich-Edwards, JW, Stampfer, MJ, Manson, JE, Rosner, B, Hankinson, SE, Colditz, GA et al. Birth weight and risk of cardiovascular disease in a cohort of women followed up since 1976. *Brit.Med.J.* (1997) 315 396-400.

10. Forsen, T, Eriksson, JG, Tuomilehto, J, Teramo, K, Osmond, C, and Barker, DJ. Mother's weight in pregnancy and coronary heart disease in a cohort of Finnish men: follow up study. *Brit.Med.J.* (1997) 315 837-840.

11. Stein, CE, Fall, CHD, Kumaran, K, Osmond, C, Cox, V, and Barker, DJP. Fetal growth and coronary heart disease in South India. *Lancet* (1996) 348 1269-1273.

12. Huxley, RR, Shiell, AW, and Law, CM. The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. *J.Hypertens.* (2000) 18 815-831.

13. Huxley, R, Neil, A, and Collins, R. Unravelling the fetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? *Lancet* (2002) 360 659-665.

14. Barker, DJP, Martyn, CN, Osmond, C, Hales, CN, and Fall, CHD. Growth in utero and serum cholesterol concentrations in adult life. *Brit.Med.J.* (1993) 307 1524-1527.

15. Fall, CHD, Osmond, C, Barker, DJP, Clark, PMS, Hales, CN, Stirling, Y et al. Fetal and infant growth and cardiovascular risk factors in women. *Brit.Med.J.* (1995) 310 428-432.

16. Hales, CN, Barker, DJP, Clark, PMS, Cox, C, Fall, C, Osmond, C et al. Fetal and infant growth and impaired glucose tolerance at age 64 years. *Brit.Med.J.* (1991) 303 1019-1022.

17. Newsome, CA, Shiell, AW, Fall, CHD, Phillips, DIW, Shier, R, and Law, CM. Is birthweight related to glucose and insulin metabolism? - a systematic review. *Diabet.Med.* (2003) 20 339-348.

18. Reaven, GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* (1988) 37 1595-1607.

19. Barker, DJP, Hales, CN, Fall, CHD, Osmond, C, Phipps, K, and Clark, PMS. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* (1993) 36 62-67.

20. Byberg, L, McKeigue, PM, Zethelius, B, and Lithell, HO. Birth weight and the insulin resistance syndrome: association of low birth weight with truncal obesity and raised plasminogen activator inhibitor-1 but not with abdominal obesity or plasma lipid disturbances. *Diabetologia* (2000) 43 54-60.

21. Valdez, R, Athens, MA, Thompson, GH, Bradshaw, BS, and Stern, MP. Birthweight and adult health outcomes in a biethnic population in the USA. *Diabetologia* (1994) <u>37</u> 624-631.

22. McKeigue, PM, Lithell, HO, and Leon, DA. Glucose tolerance and resistance to insulin-stimulated glucose uptake in men aged 70 years in relation to size at birth. *Diabetologia* (1998) <u>41</u> 1133-1138.

23. Phillips, DIW, Barker, DJP, Hales, CN, Hirst, S, and Osmond, C. Thinness at birth and insulin resistance in adult life. *Diabetologia* (1994) <u>37</u> 150-154.

24. Flanagan, DE, Moore, VM, Godsland, IF, Cockington, RA, Robinson, JS, and Phillips, DI. Fetal growth and the physiological control of glucose tolerance in adults: a minimal model analysis. *Am.J.Physiol.Endocrinol.Metab.* (2000) <u>278</u> E700-E706.

25. Javaid, MK and Cooper, C. Prenatal and childhood influences on osteoporosis. *Best Pract.Res.Clin.Endocrinol.Metab.* (2002) <u>16</u> 349-367.

26. Thompson, C, Syddall, H, Rodin, I, Osmond, C, and Barker, DJ. Birth weight and the risk of depressive disorder in late life. *Br.J.Psychiatry* (2001) <u>179</u> 450-455.

27. Barker, DJP. Mothers, babies and health in later life. Churchill Livingstone, Edinburgh. 2nd Edition. (1998).

28. Hattersley, AT and Tooke, JE. The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. *Lancet* (1999) 353 1789-1792.

29. Poulsen, P, Vaag, AA, Kyvik, KO, Moller, JD, and Beck-Nielsen, H. Low birth weight is associated with NIDDM in discordant monozygotic and dizygotic twin pairs. *Diabetologia* (1997) 40 439-446.

30. Diamond, J. Pearl Harbor and the emperor's physiologists. *Natural History* (1991) 12 2-7.

31. Woodall, SM, Johnston, BM, Breier, BH, and Gluckman, PD. Chronic maternal undernutrition in the rat leads to delayed postnatal growth and elevated blood pressure of offspring. *Pediatr.Res.* (1996) 40 438-443.

32. Kwong, WY, Wild, AE, Roberts, P, Willis, AC, and Fleming, TP. Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. *Development* (2000) 127 4195-4202.

33. Ozaki, T, Nishina, H, Hanson, MA, and Poston, L. Dietary restriction in pregnant rats causes gender-related hypertension and vascular dysfunction in offspring. *J.Physiol.* (2001) 530 141-152.

34. Langley, SC and Jackson, AA. Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. *Clin.Sci.* (1994) 86 217-222.

35. Crowe, C, Dandekar, P, Fox, M, Dhingra, K, Bennet, L, and Hanson, MA. The effects of anaemia on heart, placenta and body weight, and blood pressure in fetal and neonatal rats. *J.Physiol.* (1995) <u>488</u> 515-519.

36. Khan, IY, Taylor, PD, Dekou, V, Seed, PT, Lakasing, L, Graham, D et al. Gender-linked hypertension in offspring of lard-fed pregnant rats. *Hypertension* (2003) <u>41</u> 168-175.

37. Kind, KL, Simonetta, G, Clifton, PM, Robinson, JS, and Owens, JA. Effect of maternal feed restriction on blood pressure in the adult guinea pig. *Exp.Physiol.* (2002) <u>87</u> 469-477.

38. Hawkins, P, Steyn, C, McGarrigle, HH, Calder, NA, Saito, T, Stratford, LL et al. Cardiovascular and hypothalamic-pituitary-adrenal axis development in late gestation fetal sheep and young lambs following modest maternal nutrient restriction in early gestation. *Reprod.Fertil.Dev.* (2000) <u>12</u> 443-456.

39. Edwards, LJ, Coulter, CL, Symonds, ME, and McMillen, IC. Prenatal undernutrition, glucocorticoids and the programming of adult hypertension. *Clin.Exp.Pharmacol.Physiol.* (2001) <u>28</u> 938-941.

40. Jansson, T and Lambert, GW. Effect of intrauterine growth restriction on blood pressure, glucose tolerance and sympathetic nervous system activity in the rat at 3-4 months of age. *J.Hypertens.* (1999) <u>17</u> 1239-1248.

41. Persson, E and Jansson, T. Low birth weight is associated with elevated adult blood pressure in the chronically catheterized guinea-pig. *Acta Physiol.Scand.* (1992) 145 195-196.

42. Benediktsson, R, Lindsay, RS, Noble, J, Seckl, JR, and Edwards, CR. Glucocorticoid exposure in utero: new model for adult hypertension. *Lancet* (1993) 341 339-341.

43. Dodic, M, May, CN, Wintour, EM, and Coghlan, JP. An early prenatal exposure to excess glucocorticoid leads to hypertensive offspring in sheep. *Clin.Sci.* (1998) 94 149-155.

44. Dodic, M, Hantzis, V, Duncan, J, Rees, S, Koukoulas, I, Johnson, K et al. Programming effects of short prenatal exposure to cortisol. *FASEB* (2002) 16 1017-1026.

45. Ozanne, SE and Hales, CN. The long-term consequences of intra-uterine protein malnutrition for glucose metabolism. *Proc.Nutr.Soc.* (1999) 58 615-619.

46. Langley-Evans, SC. Fetal programming of cardiovascular function through exposure to maternal undernutrition. *Proc.Nutr.Soc.* (2001) 60 505-513.

47. Kind, KL, Clifton, PM, Grant, PA, Owens, PC, Sohlstrom, A, Roberts, CT et al. Effect of maternal feed restriction during pregnancy on glucose tolerance in the adult guinea pig. *Am.J.Physiol.Regul.Integr.Comp.Physiol.* (2003) 284 R140-R152.

48. Lindsay, RS, Lindsay, RM, Waddell, B, and Seckl, JR. Prenatal glucocorticoid exposure leads to offspring hyperglycaemia in the rat: studies with the 11β-

hydroxysteroid dehydrogenase inhibitor carbenoxolone. *Diabetologia* (1996) <u>39</u> 1299-1305.

49. Nyirenda, MJ, Lindsay, RS, Kenyon, CJ, Burchell, A, and Seckl, JR. Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. *J.Clin.Invest.* (1998) <u>101</u> 2174-2181.

50. Dodic, M, Moritz, K, Koukoulas, I, and Wintour, EM. Programmed hypertension: kidney, brain or both? *Trends Endocrinol.Metab.* (2002) <u>13</u> 403-408.

51. Green, LR. Programming of endocrine mechanisms of cardiovascular control and growth. *J.Soc.Gynecol.Investig.* (2001) <u>8</u> 57-68.

52. Andrews, RC and Walker, BR. Glucocorticoids and insulin resistance: old hormones, new targets. *Clin.Sci.* (1999) <u>96</u> 513-523.

53. Herman, JP and Cullinan, WE. Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends Neurosci.* (1997) <u>20</u> 78-84.

54. Heyns, W and Coolens, JL. Physiology of corticosteroid-binding globulin in humans. *Ann.N.Y.Acad.Sci.* (1988) <u>538</u> 122-129.

55. Walker, BR, Campbell, JC, Williams, BC, and Edwards, CR. Tissue-specific distribution of the NAD(+)-dependent isoform of 11 beta-hydroxysteroid dehydrogenase. *Endocrinology* (1992) <u>131</u> 970-972.

56. Albiston, AL, Obeyesekere, VR, Smith, RE, and Krozowski, ZS. Cloning and tissue

distribution of the human 11 beta-hydroxysteroid dehydrogenase type 2 enzyme.

Mol.Cell.Endocrinol. (1994) 105 R11-R17.

57. Mason, JW. A review of psychoendocrine research on the pituitary-adrenal cortical

system. *Psychosom.Med.* (1968) 30 576-607.

58. De Kloet, ER. Brain corticosteroid receptor balance and homeostatic control.

Front.Neuroendocrinol. (1991) 12 95-164.

59. Fisher, DA. Williams Textbook of Endocrinology. Saunders, Philadelphia. 9th Edition.

(1998) 1279-1281.

60. Matthews, SG and Challis, JR. Regulation of CRH and AVP mRNA in the developing

ovine hypothalamus: effects of stress and glucocorticoids. *Am.J.Physiol.* (1995) 268

E1096-E1107.

61. Chrousos, GP. The role of stress and the hypothalamic-pituitary-adrenal axis in the

pathogenesis of the metabolic syndrome: neuro-endocrine and target tissue-related

causes. *Int.J.Obes.Relat.Metab.Disord.* (2000) 24 (Supplement 2) S50-S55.

62. Filipovsky, J, Ducimetiere, P, Eschwege, E, Richard, JL, Rosselin, G, and Claude, JR.

The relationship of blood pressure with glucose, insulin, heart rate, free fatty acids and

plasma cortisol levels according to degree of obesity in middle-aged men. *J.Hypertens.*

(1996) 14 229-235.

63. Walker, BR, Soderberg, S, Lindahl, B, and Olsson, T. Independent effects of obesity and cortisol in predicting cardiovascular risk factors in men and women. *J.Intern.Med.* (2000) 247 198-204.

64. Stolk, RP, Lamberts, SW, de Jong, FH, Pols, HA, and Grobbee, DE. Gender differences in the associations between cortisol and insulin in healthy subjects. *J.Endocrinol.* (1996) 149 313-318.

65. Rosmond, R, Dallman, MF, and Björntorp, P. Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. *J.Clin.Endocrinol.Metab.* (1998) 83 1853-1859.

66. Ward, AMV, Fall, CHD, Stein, CE, Kumaran, K, Veena, SR, Wood, PJ et al. Cortisol and the metabolic syndrome in South Asians. *Clin.Endocrinol.(Oxf)* (2003) 58 500-505.

67. Phillips, DIW, Bennett, F, Wilks, R, Thame, M, Boyne, M, and Forrester, T. The role of maternal size and adiposity in programming offspring blood pressure and HPA activity. *Pediatr.Res.* (2003) 53 (Supplement) 31A (Abstract).

68. Hess, JL, Denenberg, VH, Zarrow, MX, and Pfeifer, WD. Modification of the corticosterone response curve as a function of handling in infancy. *Physiol.Behav.* (1969) 4 109-112.

69. Levine, S. Maternal and environmental influences on the adrenocortical response to stress in weanling rats. *Science* (1957) 156 258-260.

70. Meaney, MJ, Aitken, DH, Viau, V, Sharma, S, and Sarrieau, A. Neonatal handling alters adrenocortical negative feedback sensitivity and hippocampal type II glucocorticoid receptor binding in the rat. *Neuroendocrinology* (1989) 50 597-604.

71. Plotsky, PM and Meaney, MJ. Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. *Brain Res.Mol.Brain Res.* (1993) 18 195-200.

72. Henry, C, Kabbaj, M, Simon, H, Le Moal, M, and Maccari, S. Prenatal stress increases the hypothalamo-pituitary-adrenal axis response in young and adult rats. *J.Neuroendocrinol.* (1994) 6 341-345.

73. Weinstock, M, Matlina, E, Maor, GI, Rosen, H, and McEwen, B. Prenatal stress selectively alters the reactivity of the hypothalamic-pituitary-adrenal system in the female rat. *Brain Res.* (1992) 595 195-200.

74. Nelson, LR, Taylor, AN, Lewis, JW, Poland, RE, Redei, E, and Branch, BJ. Pituitary-adrenal responses to morphine and footshock stress are enhanced following prenatal alcohol exposure. *Alcohol Clin.Exp.Res.* (1986) 10 397-402.

75. Reul, JM, Stec, I, Wiegers, GJ, Labeur, MS, Linthorst, AC, Arzt, E et al. Prenatal immune challenge alters the hypothalamic-pituitary-adrenocortical axis in adult rats. *J.Clin.Invest.* (1994) 93 2600-2607.

76. Weinstock, M. Does prenatal stress impair coping and regulation of hypothalamic-pituitary-adrenal axis? *Neurosci.Biobehav.Rev.* (1997) 21 1-10.

77. McCormick, CM, Smythe, JW, Sharma, S, and Meaney, MJ. Sex-specific effects of prenatal stress on hypothalamic-pituitary-adrenal responses to stress and brain glucocorticoid receptor density in adult rats. *Dev.Brain Res.* (1995) <u>84</u> 55-61.

78. Kaiser, S and Sachser, N. The social environment during pregnancy and lactation affects the female offsprings' endocrine status and behaviour in guinea pigs. *Physiol.Behav.* (1998) <u>63</u> 361-366.

79. Kaiser, S and Sachser, N. Social stress during pregnancy and lactation affects in guinea pigs the male offsprings' endocrine status and infantilizes their behaviour. *Psychoneuroendocrinology* (2001) <u>26</u> 503-519.

80. Clarke, AS, Wittwer, DJ, Abbott, DH, and Schneider, ML. Long-term effects of prenatal stress on HPA axis activity in juvenile rhesus monkeys. *Dev.Psychobiol.* (1994) <u>27</u> 257-269.

81. Schneider, ML, Roughton, EC, Koehler, AJ, and Lubach, GR. Growth and development following prenatal stress exposure in primates: an examination of ontogenetic vulnerability. *Child Dev.* (1999) <u>70</u> 263-274.

82. Langley-Evans, SC. Hypertension induced by foetal exposure to a maternal low-protein diet, in the rat, is prevented by pharmacological blockade of maternal glucocorticoid synthesis. *J.Hypertens.* (1997) <u>15</u> 537-544.

83. Langley-Evans, SC, Gardner, DS, and Jackson, AA. Maternal protein restriction influences the programming of the rat hypothalamic-pituitary-adrenal axis. *J.Nutr.* (1996) <u>126</u> 1578-1585.

84. Bertram, C, Trowern, AR, Copin, N, Jackson, AA, and Whorwood, CB. The maternal diet during pregnancy programs altered expression of the glucocorticoid receptor and type 2 11beta-hydroxysteroid dehydrogenase: potential molecular mechanisms underlying the programming of hypertension in utero. *Endocrinology* (2001) <u>142</u> 2841-2853.

85. Lesage, J, Dufourny, L, Laborie, C, Bernet, F, Blondeau, B, Avril, I et al. Perinatal malnutrition programs sympathoadrenal and hypothalamic-pituitary-adrenal axis responsiveness to restraint stress in adult male rats. *J.Neuroendocrinol.* (2002) <u>14</u> 135-143.

86. Dobbing, J and Sands, J. Comparative aspects of the brain growth spurt. *Early Hum.Dev.* (1979) <u>3</u> 79-83.

87. Lingas, R, Dean, F, and Matthews, SG. Maternal nutrient restriction (48hr) modifies brain corticosteroid receptor expression and endocrine function in the fetal guinea pig. *Brain Res.* (1999) <u>846</u> 236-242.

88. Lingas, RI and Matthews, SG. A short period of maternal nutrient restriction in late gestation modifies pituitary-adrenal function in adult guinea pig offspring. *Neuroendocrinology* (2001) <u>73</u> 302-311.

89. Hawkins, P, Steyn, C, McGarrigle, HH, Saito, T, Ozaki, T, Stratford, LL et al. Effect of maternal nutrient restriction in early gestation on development of the hypothalamic-pituitary-adrenal axis in fetal sheep at 0.8-0.9 of gestation. *J.Endocrinol.* (1999) <u>163</u> 553-561.

90. Hawkins, P, Steyn, C, McGarrigle, HH, Saito, T, Ozaki, T, Stratford, LL et al. Effect of maternal nutrient restriction in early gestation on responses of the hypothalamic-pituitary-adrenal axis to acute isocapnic hypoxaemia in late gestation fetal sheep. *Exp.Physiol.* (2000) <u>85</u> 85-96.

91. Whorwood, CB, Firth, KM, Budge, H, and Symonds, ME. Maternal undernutrition during early to midgestation programs tissue-specific alterations in the expression of the glucocorticoid receptor, 11beta-hydroxysteroid dehydrogenase isoforms, and type 1 angiotensin ii receptor in neonatal sheep. *Endocrinology* (2001) <u>142</u> 2854-2864.

92. Lesage, J, Blondeau, B, Grino, M, Breant, B, and Dupouy, JP. Maternal undernutrition during late gestation induces fetal overexposure to glucocorticoids and intrauterine growth retardation, and disturbs the hypothalamo-pituitary adrenal axis in the newborn rat. *Endocrinology* (2001) <u>142</u> 1692-1702.

93. Barbazanges, A, Piazza, PV, Le Moal, M, and Maccari, S. Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. *J.Neurosci.* (1996) <u>16</u> 3943-3949.

94. Levitt, NS, Lindsay, RS, Holmes, MC, and Seckl, JR. Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and

elevates blood pressure in the adult offspring of rats. *Neuroendocrinology* (1996) <u>64</u> 412-418.

95. Welberg, LA, Seckl, JR, and Holmes, MC. Prenatal glucocorticoid programming of brain corticosteroid receptors and corticotrophin-releasing hormone: possible implications for behaviour. *Neuroscience* (2001) <u>104</u> 71-79.

96. Muneoka, K, Mikuni, M, Ogawa, T, Kitera, K, Kamei, K, Takigawa, M et al. Prenatal dexamethasone exposure alters brain monoamine metabolism and adrenocortical response in rat offspring. *Am.J.Physiol.* (1997) <u>273</u> R1669-R1675.

97. Kraft, N, Hodgson, AJ, and Funder, JW. *Glucocorticoid receptor and effector mechanisms: a comparison of the corticosensitive mouse with the corticoresistant guinea pig. Endocrinology* (1979) <u>104</u> 344-349.

98. Dean, F and Matthews, SG. Maternal dexamethasone treatment in late gestation alters *glucocorticoid and mineralocorticoid receptor mRNA in the fetal guinea pig brain. Brain Res.* (1999) <u>846</u> 253-259.

99. Liu, L, Li, A, and Matthews, SG. Maternal glucocorticoid treatment programs HPA regulation in adult offspring: sex-specific effects. *Am.J.Physiol.Endocrinol.Metab.* (2001) <u>280</u> E729-E739.

100. Sloboda, DM, Moss, TJ, Gurrin, LC, Newnham, JP, and Challis, JR. The effect of prenatal betamethasone administration on postnatal ovine hypothalamic-pituitary-adrenal function. *J.Endocrinol.* (2002) <u>172</u> 71-81.

101. Dodic, M, Peers, A, Moritz, K, Hantzis, V, and Wintour, EM. No evidence for HPA reset in adult sheep with high blood pressure due to short prenatal exposure to dexamethasone. *Am.J.Physiol.Regul.Integr.Comp.Physiol.* (2002) 282 R343-R350.

102. Uno, H, Eisele, S, Sakai, A, Shelton, S, Baker, E, DeJesus, O et al. Neurotoxicity of glucocorticoids in the primate brain. *Horm.Behav.* (1994) 28 336-348.

103. Lindsay, RS, Lindsay, RM, Edwards, CR, and Seckl, JR. Inhibition of 11-beta-hydroxysteroid dehydrogenase in pregnant rats and the programming of blood pressure in the offspring. *Hypertension* (1996) 27 1200-1204.

104. Welberg, LA, Seckl, JR, and Holmes, MC. Inhibition of 11beta-hydroxysteroid dehydrogenase, the foeto-placental barrier to maternal glucocorticoids, permanently programs amygdala GR mRNA expression and anxiety-like behaviour in the offspring. *Eur.J.Neurosci.* (2000) 12 1047-1054.

105. Stewart, PM, Rogerson, FM, and Mason, JI. Type 2 11 beta-hydroxysteroid dehydrogenase messenger ribonucleic acid and activity in human placenta and fetal membranes: its relationship to birth weight and putative role in fetal adrenal steroidogenesis. *J.Clin.Endocrinol.Metab.* (1995) 80 885-890.

106. Langley-Evans, SC, Phillips, GJ, Benediktsson, R, Gardner, DS, Edwards, CR, Jackson, AA et al. Protein intake in pregnancy, placental glucocorticoid metabolism and the programming of hypertension in the rat. *Placenta* (1996) 17 169-172.

107. Doyle, LW, Ford, GW, Davis, NM, and Callanan, C. Antenatal corticosteroid therapy and blood pressure at 14 years of age in preterm children. *Clin.Sci.* (2000) 98 137-142.

108. Trautman, PD, Meyer-Bahlburg, HF, Postelnek, J, and New, MI. Effects of early prenatal dexamethasone on the cognitive and behavioral development of young children: results of a pilot study. *Psychoneuroendocrinology* (1995) 20 439-449.

109. Herrick, K, Phillips, DIW, Haselden, S, Sheill, AW, Campbell-Brown, M, and Godfrey, KM. Maternal consumption of a high meat, low carbohydrate diet in late pregnancy: relation to adult cortisol concentrations in the offspring. *J.Clin.Endocrinol.Metab.* (2003) 88 3554-3560.

110. Phillips, DIW, Barker, DJP, Fall, CHD, Seckl, JR, Whorwood, CB, Wood, PJ et al. Elevated plasma cortisol concentrations: a link between low birthweight and the insulin resistance syndrome? *J.Clin.Endocrinol.Metab.* (1998) 83 757-760.

111. Phillips, DIW, Walker, BR, Reynolds, RM, Flanagan, DE, Wood, PJ, Osmond, C et al. Low birthweight predicts elevated plasma cortisol concentrations in adults from three populations. *Hypertension* (2000) 35 1301-1306.

112. Clark, PM, Hindmarsh, PC, Shiell, AW, Law, CM, Honour, JW, and Barker, DJP. Size at birth and adrenocortical function in childhood. *Clin.Endocrinol.(Oxf)* (1996) 45 721-726.

113. Kajantie, E, Phillips, DI, Andersson, S, Barker, DJ, Dunkel, L, Forsen, T et al. Size at birth, gestational age and cortisol secretion in adult life: foetal programming of both hyper- and hypocortisolism? *Clin.Endocrinol.(Oxf)* (2002) <u>57</u> 635-641.

114. Ward, AMV, Fall, CHD, Kumaran, K, Phillips, DIW, and Wood, PJ. Programming of the hypothalamic-pituitary-adrenal axis differs in Asian and Caucasian populations. *Pediatr.Res.* (2001) <u>50</u> (Supplement) 38A (Abstract).

115. Fall, CH, Dennison, E, Cooper, C, Pringle, J, Kellingray, SD, and Hindmarsh, P. Does birth weight predict adult serum cortisol concentrations? Twenty-four-hour profiles in the United kingdom 1920-1930 Hertfordshire Birth Cohort. *J.Clin.Endocrinol.Metab.* (2002) <u>87</u> 2001-2007.

116. Dahlgren, J, Boguszewski, M, Rosberg, S, and Albertsson-Wikland, K. Adrenal steroid hormones in short children born small for gestational age. *Clin.Endocrinol.(Oxf)* (1998) <u>49</u> 353-361.

117. Reynolds, RM, Walker, BR, Syddall, HE, Andrew, R, Wood, PJ, Whorwood, CB et al. Altered control of cortisol secretion in adult men with low birthweight and cardiovascular risk factors. *J.Clin.Endocrinol.Metab.* (2001) <u>86</u> 245-250.

118. Reynolds, RM, Walker, BR, Syddall, HE, Andrews, R, Wood, PJ, and Phillips, DIW. Activation of the hypothalamic-pituitary-adrenal axis in adults of low birthweight: similar increased activity in men and women. *Pediatr.Res.* (2003) <u>53</u> (Supplement) 39A (Abstract).

119. Levitt, NS, Lambert, EV, Woods, D, Hales, CN, Andrew, R, and Seckl, JR. Impaired Glucose Tolerance and Elevated Blood Pressure in Low Birth Weight, Nonobese, Young South African Adults: Early Programming of Cortisol Axis. *J.Clin.Endocrinol.Metab.* (2000) 85 4611-4618.

120. Morrison, SF, Ramamurthy, S, and Young, JB. Reduced rearing temperature augments responses in sympathetic outflow to brown adipose tissue. *J.Neurosci.* (2000) 20 9264-9271.

121. Young, JB. Effects of neonatal handling on sympathoadrenal activity and body composition in adult male rats. *Am.J.Physiol.Regul.Integr.Comp.Physiol.* (2000) 279 R1745-R1752.

122. Young, JB and Morrison, SF. Effects of fetal and neonatal environment on sympathetic nervous system development. *Diabetes Care* (1998) 21 Suppl 2 B156-B160.

123. Petry, CJ, Dorling, MW, Wang, CL, Pawlak, DB, and Ozanne, SE. Catecholamine levels and receptor expression in low protein rat offspring. *Diabet.Med.* (2000) 17 848-853.

124. Weinstock, M, Poltyrev, T, Schorer-Apelbaum, D, Men, D, and McCarty, R. Effect of prenatal stress on plasma corticosterone and catecholamines in response to footshock in rats. *Physiol.Behav.* (1998) 64 439-444.

125. Nyirenda, MJ, Welberg, LA, and Seckl, JR. Programming hyperglycaemia in the rat through prenatal exposure to glucocorticoids-fetal effect or maternal influence? *J.Endocrinol.* (2001) 170 653-660.

126. Simonetta, G, Rourke, AK, Owens, JA, Robinson, JS, and McMillen, IC. Impact of placental restriction on the development of the sympathoadrenal system. *Pediatr.Res.* (1997) 42 805-811.

127. McMillen, IC, Adams, MB, Ross, JT, Coulter, CL, Simonetta, G, Owens, JA et al. Fetal growth restriction: adaptations and consequences. *Reproduction* (2001) 122 195-204.

128. Robinson, SM, Wheeler, T, Hayes, MC, Barker, DJP, and Osmond, C. Fetal heart rate and intrauterine growth. *Br.J.Obstet.Gynaecol.* (1991) 98 1223-1227.

129. Spassov, L, Curzi-Dascalova, L, Clairambault, J, Kauffmann, F, Eiselt, M, Medigue, C et al. Heart rate and heart rate variability during sleep in small-for-gestational age newborns. *Pediatr.Res.* (1994) 35 500-505.

130. Phillips, DI and Barker, DJ. Association between low birthweight and high resting pulse in adult life: is the sympathetic nervous system involved in programming the insulin resistance syndrome? *Diabet.Med.* (1997) 14 673-677.

131. Longo-Mbenza, B, Ngiyulu, R, Bayekula, M, Vita, EK, Nkiabungu, FB, Seghers, KV et al. Low birth weight and risk of hypertension in African school children. *J.Cardiovasc.Risk* (1999) 6 311-314.

132. Tenhola, S, Martikainen, A, Rahiala, E, Parviainen, M, Halonen, P, and Voutilainen, R. Increased adrenocortical and adrenomedullary hormonal activity in 12-year-old children born small for gestational age. *J.Pediatr.* (2002) <u>141</u> 477-482.

133. Chrousos, GP and Gold, PW. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA* (1992) <u>267</u> 1244-1252.

134. Pacak, K, Palkovits, M, Kopin, IJ, and Goldstein, DS. Stress-induced norepinephrine release in the hypothalamic paraventricular nucleus and pituitary-adrenocortical and sympathoadrenal activity: in vivo microdialysis studies. *Front.Neuroendocrinol.* (1995) <u>16</u> 89-150.

135. Kvetnansky, R, Pacak, K, Fukuhara, K, Viskupic, E, Hiremagalur, B, Nankova, B et al. Sympathoadrenal system in stress. Interaction with the hypothalamic-pituitary-adrenal system. *Ann.N.Y.Acad.Sci.* (1995) <u>771</u> 131-158.

136. Haidan, A, Bornstein, SR, Liu, Z, Walsh, LP, Stocco, DM, and Ehrhart-Bornstein, M. Expression of adrenocortical steroidogenic acute regulatory (StAR) protein is influenced by chromaffin cells. *Mol.Cell.Endocrinol.* (2000) <u>165</u> 25-32.

137. Board, F, Persky, H, and Hamburg, DA. Psychological stress and endocrine functions. *Psychosom.Med.* (1956) <u>18</u> 324-333.

138. Carroll, BJ, Feinberg, M, Greden, JF, Tarika, J, Albala, AA, Haskett, RF et al. A specific laboratory test for the diagnosis of melancholia. Standardization, validation, and clinical utility. *Arch.Gen.Psychiatry* (1981) <u>38</u> 15-22.

139. Rubin, RT, Poland, RE, Lesser, IM, Winston, RA, and Blodgett, AL. Neuroendocrine aspects of primary endogenous depression. I. Cortisol secretory dynamics in patients and matched controls. *Arch.Gen.Psychiatry* (1987) 44 328-336.

140. Gold, PW, Goodwin, FK, and Chrousos, GP. Clinical and biochemical manifestations of depression. Relation to the neurobiology of stress (1). *N.Engl.J.Med.* (1988) 319 348-353.

141. Vale, W, Speiss, J, Rivier, C, and Rivier, J. Characterisation of a 41-residue ovine hypothalmic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* (1981) 213 1394-1397.

142. Arborelius, L, Owens, MJ, Plotsky, PM, and Nemeroff, CB. The role of corticotropin-releasing factor in depression and anxiety disorders. *J.Endocrinol.* (1999) 160 1-12.

143. Nemeroff, CB, Widerlov, E, Bissette, G, Walleus, H, Karlsson, I, Eklund, K et al. Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* (1984) 226 1342-1344.

144. Nemeroff, CB, Owens, MJ, Bissette, G, Andorn, AC, and Stanley, M. Reduced corticotropin releasing factor binding sites in the frontal cortex of suicide victims. *Arch.Gen.Psychiatry* (1988) 45 577-579.

145. Kling, MA, Roy, A, Doran, AR, Calabrese, JR, Rubinow, DR, Whitfield, HJJ et al. Cerebrospinal fluid immunoreactive corticotropin-releasing hormone and

adrenocorticotropin secretion in Cushing's disease and major depression: potential clinical implications. *J.Clin.Endocrinol.Metab.* (1991) 72 260-271.

146. Holsboer, F, von Bardeleben, U, Gerken, A, Stalla, GK, and Muller, OA. Blunted corticotropin and normal cortisol response to human corticotropin-releasing factor in depression [letter]. *N.Engl.J.Med.* (1984) 311 1127

147. Gold, PW, Loriaux, DL, Roy, A, Kling, M, Calabrese, JR, Kellner, CH et al. Responses to corticotropin-releasing hormone in the hypercortisolism of depression and Cushing's disease. Pathophysiologic and diagnostic implications. *N.Engl.J.Med.* (1986) 314 1329-1335.

148. Raadsheer, FC, Hoogendijk, WJ, Stam, FC, Tilders, FJ, and Swaab, DF. Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. *Neuroendocrinology* (1994) 60 436-444.

149. Holsboer, F, von Bardeleben, U, Wiedemann, K, Muller, OA, and Stalla, GK. Serial assessment of corticotropin-releasing hormone response after dexamethasone in depression. Implications for pathophysiology of DST nonsuppression. *Biol.Psychiatry* (1987) 22 228-234.

150. von Bardeleben, U and Holsboer, F. Effect of age on the cortisol response to human corticotropin-releasing hormone in depressed patients pretreated with dexamethasone. *Biol.Psychiatry* (1991) 29 1042-1050.

151. Deuschle, M, Schweiger, U, Gotthardt, U, Weber, B, Korner, A, Schmider, J et al. The combined dexamethasone/corticotropin-releasing hormone stimulation test is more closely associated with features of diurnal activity of the hypothalamo-pituitary-adrenocortical system than the dexamethasone suppression test. *Biol.Psychiatry* (1998) <u>43</u> 762-766.

152. Heuser, I, Yassouridis, A, and Holsboer, F. The combined dexamethasone/CRH test: a refined laboratory test for psychiatric disorders. *J.Psychiatr.Res.* (1994) <u>28</u> 341-356.

153. Meijer, OC, de Lange, EC, Breimer, DD, de Boer, AG, Workel, JO, and De Kloet, ER. Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdr1A P-glycoprotein knockout mice. *Endocrinology* (1998) <u>139</u> 1789-1793.

154. Keck, ME, Wigger, A, Welt, T, Muller, MB, Gesing, A, Reul, JM et al. Vasopressin mediates the response of the combined dexamethasone/CRH test in hyper-anxious rats: implications for pathogenesis of affective disorders. *Neuropsychopharmacology* (2002) <u>26</u> 94-105.

155. Heuser, IJ, Gotthardt, U, Schweiger, U, Schmider, J, Lammers, CH, Dettling, M et al. Age-associated changes of pituitary-adrenocortical hormone regulation in humans: importance of gender. *Neurobiol.Aging* (1994) <u>15</u> 227-231.

156. Duclos, M, Corcuff, JB, Pehourcq, F, and Tabarin, A. Decreased pituitary sensitivity to glucocorticoids in endurance-trained men. *Eur.J.Endocrinol.* (2001) <u>144</u> 363-368.

157. Huizenga, NA, Koper, JW, de Lange, P, Pols, HA, Stolk, RP, Grobbee, DE et al.
Interperson variability but intraperson stability of baseline plasma cortisol
concentrations, and its relation to feedback sensitivity of the hypothalamo-pituitary-
adrenal axis to a low dose of dexamethasone in elderly individuals.
J.Clin.Endocrinol.Metab. (1998) 83 47-54.

158. Coste, J, Strauch, G, Letrait, M, and Bertagna, X. Reliability of hormonal levels for
assessing the hypothalamic-pituitary-adrenocortical system in clinical pharmacology.
Br.J.Clin.Pharmacol. (1994) 38 474-479.

159. Spath-Schwalbe, E, Scholler, T, Kern, W, Fehm, HL, and Born, J. Nocturnal
adrenocorticotropin and cortisol secretion depends on sleep duration and decreases in
association with spontaneous awakening in the morning. *J. Clin.Endocrinol.Metab.*
(1992) 75 1431-1435.

160. Vining, RF, McGinley, RA, and Symons, RG. Hormones in saliva: mode of entry and
consequent implications for clinical interpretation. *Clin.Chem.* (1983) 29 1752-1756.

161. Kahn, JP, Rubinow, DR, Davis, CL, Kling, M, and Post, RM. Salivary cortisol: a
practical method for evaluation of adrenal function. *Biol.Psychiatry* (1988) 23 335-349.

162. Pruessner, JC, Wolf, OT, Hellhammer, DH, Buske-Kirschbaum, A, von Auer, K, Jobst,
S et al. Free cortisol levels after awakening: a reliable biological marker for the
assessment of adrenocortical activity. *Life Sci.* (1997) 61 2539-2549.

163. Schulz, P, Kirschbaum, C, Pruessner, JC, and Hellhammer, DH. Increased free cortisol after awakening in chronically stressed individuals due to work overload. *Stress Med.* (1998) 14 91-97.

164. Pruessner, JC, Hellhammer, DH, and Kirschbaum, C. Burnout, perceived stress, and cortisol responses to awakening. *Psychosom.Med.* (1999) 61 197-204.

165. Edwards, S, Clow, A, Evans, P, and Hucklebridge, F. Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity. *Life Sci.* (2001) 68 2093-2103.

166. Zigmond, AS and Snaith, RP. The hospital anxiety and depression scale. *Acta.Psychiatr.Scand.* (1983) 67 361-370.

167. Durnin, JV and Womersley, J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br.J.Nutr.* (1974) 32 77-97.

168. von Bardeleben, U and Holsboer, F. Human corticotropin releasing hormone: clinical studies in patients with affective disorders, alcoholism, panic disorder and in normal controls. *Prog.Neuropsychopharmacol.Biol.Psychiatry* (1988) 12 Suppl S165-S187.

169. Trainer, PJ, Faria, M, Newell-Price, J, Browne, P, Kopelman, P, Coy, DH et al. A comparison of the effects of human and ovine corticotropin-releasing hormone on the pituitary-adrenal axis. *J.Clin.Endocrinol.Metab.* (1995) 80 412-417.

170. Schürmeyer, TH, Avgerinos, PC, Gold, PW, Gallucci, WT, Tomai, TP, Cutler, GB, Jr. et al. Human corticotropin-releasing factor in man: pharmacokinetic properties and dose-response of plasma adrenocorticotropin and cortisol secretion. *J.Clin.Endocrinol.Metab.* (1984) 59 1103-1108.

171. Linton, EA and Lowry, PJ. Corticotrophin releasing factor in man and its measurement: a review. *Clin.Endocrinol.(Oxf)* (1989) 31 225-249.

172. Lytras, N, Grossman, A, Perry, L, Tomlin, S, Wass, JAH, Coy, DH et al. Corticotrophin releasing factor: responses in normal subjects and patients with disorders of the hypothalamus and pituitary. *Clin.Endocrinol.(Oxf)* (1984) 20 71-84.

173. Morineau, G, Boudi, A, Barka, A, Gourmelen, M, Degeilh, F, Hardy, N et al. Radioimmunoassay of cortisone in serum, urine, and saliva to assess the status of the cortisol-cortisone shuttle. *Clin.Chem.* (1997) 43 1397-1407.

174. Moore, A, Aitken, R, Burke, C, Gaskell, S, Groom, G, Holder, G et al. Cortisol assays: guidelines for the provision of a clinical biochemistry service. *Ann.Clin.Biochem.* (1985) 22 (Pt 5) 435-454.

175. Sobey, WJ, Beer, SF, Carrington, CA, Clark, PM, Frank, BH, Gray, IP et al. Sensitive and specific two-site immunoradiometric assays for human insulin, proinsulin, 65-66 split and 32-33 split proinsulins. *Biochem.J.* (1989) 260 535-541.

176. Alberti, KG and Zimmet, PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet.Med.* (1998) 15 539-553.

177. Matthews, DR, Hosker, JP, Rudenski, AS, Naylor, BA, Treacher, DF, and Turner, RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* (1985) 28 412-419.

178. Liang, KY and Zeger, SL. Longitudinal data analysis using generalized linear models. *Biometrika* (1986) 73 13-22.

179. Schmidt-Reinwald, A, Pruessner, JC, Hellhammer, DH, Federenko, I, Rohleder, N, Schürmeyer, TH et al. The cortisol response to awakening in relation to different challenge tests and a 12-hour cortisol rhythm. *Life Sci.* (1999) 64 1653-1660.

180. Kudielka, BM, Broderick, JE, and Kirschbaum, C. Compliance with saliva sampling protocols: electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects. *Psychosom.Med.* (2003) 65 313-319.

181. Kudielka, BM and Kirschbaum, C. Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. *Psychoneuroendocrinology* (2003) 28 35-47.

182. Edwards, S, Evans, P, Hucklebridge, F, and Clow, A. Association between time of awakening and diurnal cortisol secretory activity. *Psychoneuroendocrinology* (2001) 26 613-622.

183. Hellhammer, DH, Wüst, S, Wolf, J, Federenko, I, and Kirschbaum, C. Attenuated basal adrenocortical activity in adults born either preterm or low birthweight. *Pediatr.Res.* (2001) 50 (Supplement) 57A (Abstract).

184. Demitrack, MA, Dale, JK, Straus, SE, Laue, L, Listwak, SJ, Kruesi, MJ et al. Evidence for impaired activation of the hypothalamic-pituitary-adrenal axis in patients with chronic fatigue syndrome. *J.Clin.Endocrinol.Metab.* (1991) 73 1224-1234.

185. Scott, LV, Medbak, S, and Dinan, TG. Blunted adrenocorticotropin and cortisol responses to corticotropin-releasing hormone stimulation in chronic fatigue syndrome. *Acta.Psychiatr.Scand.* (1998) 97 450-457.

186. Joseph-Vanderpool, JR, Rosenthal, NE, Chrousos, GP, Wehr, TA, Skwerer, R, Kasper, S et al. Abnormal pituitary-adrenal responses to corticotropin-releasing hormone in patients with seasonal affective disorder: clinical and pathophysiological implications. *J.Clin.Endocrinol.Metab.* (1991) 72 1382-1387.

187. Welberg, LA and Seckl, JR. Prenatal stress, glucocorticoids and the programming of the brain. *J.Neuroendocrinol.* (2001) 13 113-128.

188. Kopelman, PG, Grossman, A, Lavender, P, Besser, GM, Rees, LH, and Coy, DH. The cortisol response to corticotropin-releasing factor is blunted in obesity. *Clin.Endocrinol.(Oxf)* (1988) 28 15-18.

189. Pasquali, R, Cantobelli, S, Casimirri, F, Capelli, M, Bortoluzzi, L, Flamia, R et al. The hypothalamic-pituitary-adrenal axis in obese women with different patterns of body fat distribution. *J.Clin.Endocrinol.Metab.* (1993) 77 341-346.

190. Solano, MP, Kumar, M, Fernandez, B, Jones, L, and Goldberg, RB. The pituitary response to ovine corticotropin-releasing hormone is enhanced in obese men and correlates with insulin resistance. *Horm.Metab.Res.* (2001) 33 39-43.

191. Ljung, T, Holm, G, Friberg, P, Andersson, B, Bengtsson, BA, Svensson, J et al. The activity of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in relation to waist/hip circumference ratio in men. *Obes.Res.* (2000) 8 487-495.

192. Katz, JR, Taylor, NF, Goodrick, S, Perry, L, Yudkin, JS, and Coppack, SW. Central obesity, depression and the hypothalamo-pituitary-adrenal axis in men and postmenopausal women. *Int.J.Obes.Relat.Metab.Disord.* (2000) 24 246-251.

193. Rask, E, Olsson, T, Soderberg, S, Andrew, R, Livingstone, DE, Johnson, O et al. Tissue-specific dysregulation of cortisol metabolism in human obesity. *J.Clin.Endocrinol.Metab.* (2001) 86 1418-1421.

194. Bano, G, Rodin, DA, White, A, O'Rahilly, S, and Nussey, SS. Is the defect in pro-hormone processing in Type 2 diabetes mellitus restricted to the beta cell? *Diabet.Med.* (2001) 18 17-21.

195. Björntorp, P. Neuroendocrine abnormalities in human obesity. *Metabolism* (1995) 44 38-41.

196. Ljung, T, Andersson, B, Bengtsson, BA, Bjorntorp, P, and Marin, P. Inhibition of cortisol secretion by dexamethasone in relation to body fat distribution: a dose-response study. *Obes.Res.* (1996) 4 277-282.

197. Walker, BR, Phillips, DI, Noon, JP, Panarelli, M, Andrew, R, Edwards, HV et al. Increased glucocorticoid activity in men with cardiovascular risk factors. *Hypertension* (1998) 31 891-895.

198. Nilsson, PM, Nyberg, P, and Ostergren, PO. Increased susceptibility to stress at a psychological assessment of stress tolerance is associated with impaired fetal growth. *Int.J.Epidemiol.* (2001) 30 75-80.

199. Kamarck, TW, Jennings, JR, Debski, TT, Glickman-Weiss, E, Johnson, PS, Eddy, MJ et al. Reliable measures of behaviorally-evoked cardiovascular reactivity from a PC-based test battery: results from student and community samples. *Psychophysiology* (1992) 29 17-28.

200. Manuck, SB. Cardiovascular reactivity in Cardiovascular Disease:"Once more into the breach". *Int.J.Behav.Med* (1994) 1 4-31.

201. Treiber, FA, Kamarck, T, Schneiderman, N, Sheffield, D, Kapuku, G, and Taylor, T. Cardiovascular reactivity and development of preclinical and clinical disease states. *Psychosom.Med.* (2003) 65 46-62.

202. Tonkiss, J, Trzcinska, M, Galler, JR, Ruiz-Opazo, N, and Herrera, VL. Prenatal malnutrition-induced changes in blood pressure: dissociation of stress and nonstress responses using radiotelemetry. *Hypertension* (1998) 32 108-114.

203. Biondi, M and Picardi, A. Psychological stress and neuroendocrine function in humans: the last two decades of research. *Psychother.Psychosom.* (1999) 68 114-150.

204. Kirschbaum, C, Pirke, KM, and Hellhammer, DH. The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* (1993) 28 76-81.

205. Roy, MP, Kirschbaum, C, and Steptoe, A. Psychological, cardiovascular, and metabolic correlates of individual differences in cortisol stress recovery in young men. *Psychoneuroendocrinology* (2001) 26 375-391.

206. Steptoe, A, Fieldman, G, Evans, O, and Perry, L. Cardiovascular risk and responsivity to mental stress: the influence of age, gender and risk factors. *J.Cardiovasc.Risk* (1996) 3 83-93.

207. Steptoe, A and Vogele, C. Methodology of mental stress testing in cardiovascular research. *Circulation* (1991) 83 II4-II24.

208. Boulton, TJ, Cockington, RA, Hamilton-Craig, I, Magarey, AM, and Mazumdar, J. A profile of heart disease risk factors and their relation to parents' education, fathers' occupation and family history of heart disease in 843 South Australian families: the

Adelaide Children's WHO Collaborative Study. *J.Paediatr.Child Health* (1995) <u>31</u> 200-206.

209. Moore, VM, Miller, AG, Boulton, TJ, Cockington, RA, Craig, IH, Magarey, AM et al. Placental weight, birth measurements, and blood pressure at age 8 years. *Arch.Dis.Child.* (1996) <u>74</u> 538-541.

210. Moore, VM, Cockington, RA, Ryan, P, and Robinson, JS. The relationship between birth weight and blood pressure amplifies from childhood to adulthood. *J.Hypertens.* (1999) <u>17</u> 883-888.

211. Roberts, RE and Vernon, SW. The Center for Epidemiologic Studies Depression Scale: its use in a community sample. *Am.J.Psychiatry* (1983) <u>140</u> 41-46.

212. Glover, J and Tennant, S. A social health atlas of Australia. Public Health Information Development Unit 2nd Edition. (1999) Volume 5: South Australia. 17.

213. Kamarck, TW and Lovallo, WR. Cardiovascular reactivity to psychological challenge: conceptual and measurement considerations. *Psychosom.Med.* (2003) <u>65</u> 9-21.

214. Kirschbaum, C, Kudielka, BM, Gaab, J, Schommer, NC, and Hellhammer, DH. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosom.Med.* (1999) <u>61</u> 154-162.

215. Gibson, EL, Checkley, S, Papadopoulos, A, Poon, L, Daley, S, and Wardle, J. Increased salivary cortisol reliably induced by a protein-rich midday meal. *Psychosom.Med.* (1999) 61 214-224.

216. Argyropoulos, SV, Bailey, JE, Hood, SD, Kendrick, AH, Rich, AS, Laszlo, G et al. Inhalation of 35% CO(2) results in activation of the HPA axis in healthy volunteers. *Psychoneuroendocrinology* (2002) 27 715-729.

217. IJzerman, RG, Stehouwer, CD, and Boomsma, DI. Evidence for genetic factors explaining the birth weight-blood pressure relation. Analysis in twins. *Hypertension* (2000) 36 1008-1012.

218. Taylor, PD, Mehta, N, Khan, IY, Douglas, G, Jensen, R, and Poston, L. Exaggerated cardiovascular stress responses in the hypertensive offspring of lard-fed dams. *Pediatr.Res.* (2003) 53 (Supplement) 33A (Abstract).

219. Kasprowicz, AL, Manuck, SB, Malkoff, SB, and Krantz, DS. Individual differences in behaviorally evoked cardiovascular response: temporal stability and hemodynamic patterning. *Psychophysiology* (1990) 27 605-619.

220. McCaffery, JM, Muldoon, MF, Bachen, EA, Jennings, JR, and Manuck, SB. Behaviorally-evoked plasma catecholamine response and 24-hour excretion of urinary catecholamines among cardiac and vascular reactors. *Biol.Psychol.* (2000) 52 53-69.

221. Stoney, CM, Davis, MC, and Matthews, KA. Sex differences in physiological responses to stress and in coronary heart disease: a causal link? *Psychophysiology* (1987) 24 127-131.

222. Frisk, V, Amsel, R, and Whyte, HE. The importance of head growth patterns in predicting the cognitive abilities and literacy skills of small-for-gestational-age children. *Dev.Neuropsychol.* (2002) 22 565-593.

223. Nemeroff, CB, Krishnan, KR, Reed, D, Leder, R, Beam, C, and Dunnick, NR. Adrenal gland enlargement in major depression. A computed tomographic study. *Arch.Gen.Psychiatry* (1992) 49 384-387.

224. Matthews, SG. Early programming of the hypothalamo-pituitary-adrenal axis. *Trends Endocrinol.Metab.* (2002) 13 373-380.

225. Weinstock, M. Alterations induced by gestational stress in brain morphology and behaviour of the offspring. *Prog.Neurobiol.* (2001) 65 427-451.

226. Owen, D and Matthews, SG. Glucocorticoids and sex-dependent development of brain glucocorticoid and mineralocorticoid receptors. *Endocrinology* (2003) 144 2775-2784.

227. Pariante, CM, Papadopoulos, AS, Poon, L, Checkley, SA, English, J, Kerwin, RW et al. A novel prednisolone suppression test for the hypothalamic-pituitary-adrenal axis. *Biol.Psychiatry* (2002) 51 922-930.

228. Ward, AMV, Syddall, HE, Wood, PJ, Reynolds, RM, and Phillips, DIW. Intercomparison of low dose dexamethasone, prednisolone and hydrocortisone suppression tests. *Endocrine Abstracts* (2001) 1 P141 (Abstract).

229. Eriksson, JG, Lindi, V, Uusitupa, M, Forsen, TJ, Laakso, M, Osmond, C et al. The effects of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma2 gene on insulin sensitivity and insulin metabolism interact with size at birth. *Diabetes* (2002) 51 2321-2324.

230. Bogdarina, I, Murphy, HC, Burns, SP, and Clark, AJ. Investigation of the role of epigenetic modification of the rat glucokinase gene in fetal programming. *Life Sci.* (2004) 74 1407-1415.

231. Weaver, IC, Szyf, M, and Meaney, MJ. From maternal care to gene expression: DNA methylation and the maternal programming of stress responses. *Endocr.Res.* (2002) 28 699